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(54) **NOVEL OSTEOINDUCTIVE COMPOSITIONS**
OSTEOINDUKTIVE MITTEL
NOUVELLES COMPOSITIONS OSTEOINDUCTIVES

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EP 0 313 578 B1

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Description

The present invention relates to novel proteins, processes for obtaining them and genes encoding them. These proteins are capable of inducing cartilage and bone formation.

Background

Bone is a highly specialized tissue characterized by an extensive matrix structure formed of fibrous bundles of the protein collagen, and proteoglycans, noncollagenous proteins, lipids and acidic proteins. The processes of bone formation and renewal/repair of bone tissue, which occur continuously throughout life, are performed by specialized cells. Normal embryonic long bone development is preceded by formation of a cartilage model. Bone growth is presumably mediated by "osteoblasts" (bone-forming cells), while remodeling of bone is apparently accomplished by the joint activities of bone-resorbing cells, called "osteoclasts" and osteoblasts. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g. European patent applications 148,155 and 169,016 for discussions thereof.

Brief Description of the Invention

The present invention provides novel proteins in purified form and genes encoding them. Specifically, two of the novel proteins are designated BMP-2 Class I (or BMP-2), and BMP-2 Class II (or BMP-4) wherein BMP is bone morphogenic protein. These proteins are characterized by peptide sequences the same as or substantially homologous to amino acid sequences illustrated in Tables II, III and IV below. They are capable of inducing bone formation at a predetermined site. These bone inductive factors are further characterized by biochemical and biological characteristics including activity at a concentration of 10 to 1000ng/gram of bone in an *in vivo* rat bone formation assay described below. Proteins of this invention may be encoded by the DNA sequences depicted in the Tables or by sequences capable of hybridizing thereto and coding for polypeptides with bone growth factor biological properties or other variously modified sequences demonstrating such properties.

One of the proteins of the invention is designated BMP-2 Class I (or BMP-2). It is characterized by at least a portion of a peptide sequence the same or substantially the same as that of amino acid #1 through amino acid #396 of Table III which represents the cDNA hBMP-2 Class I. This peptide sequence is encoded by the same or substantially the same DNA sequence, as depicted in nucleotide #356 through nucleotide #1543 of Table III. The human peptide sequence identified in Table III is 396 amino acids in length. hBMP-2 or related bone inductive proteins may also be characterized by at least a portion of this peptide sequence. hBMP-2 Class I is further characterized by the ability to induce bone formation.

The homologous bovine bone inductive protein of the invention designated bBMP-2 Class I (or bBMP-2), has a DNA sequence identified in Table II below which represents the genomic sequence. This bovine DNA sequence has a prospective 129 amino acid coding sequence followed by approximately 205 nucleotides (a presumptive 3' non-coding sequence). bBMP-2, Class I is further characterized by the ability to induce bone formation. A further bone inductive protein composition of the invention is designated BMP-2 Class II or BMP-4. The human protein hBMP-2 Class II (or hBMP-4) is characterized by at least a portion of the same or substantially the same peptide sequence between amino acid #1 through amino acid #408 of Table IV, which represents the cDNA of hBMP-2 Class II. This peptide sequence is encoded by at least a portion of the same or substantially the same DNA sequence as depicted in nucleotide #403 through nucleotide #1626 of Table IV. This factor is further characterized by the ability to induce bone formation.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of one or more bone growth factor polypeptides according to the invention in a pharmaceutically acceptable vehicle. These compositions may further include other therapeutically useful agents. They may also include an appropriate matrix for delivering the proteins to the site of the bone defect and for providing a structure for bone growth. These compositions may be employed in methods for treating a number of bone defects and periodontal disease. These methods, according to the invention, entail administering to a patient needing such bone formation an effective amount of at least one of the novel proteins BMP-2 Class I and BMP-2 Class-II as described herein.

Still a further aspect of the invention are DNA sequences coding on expression for a human or bovine polypeptide having the ability to induce bone formation. Such sequences include the sequence of nucleotides in a 5' to 3' direction illustrated in Tables II, III and IV. Alternatively, a DNA sequence which hybridizes under stringent conditions with the DNA sequences of Tables II, III and IV or a DNA sequence which hybridizes under non-stringent conditions with the illustrated DNA sequences and which codes on expression for a protein having at least one bone growth factor biological property are included in the present invention. Finally, allelic or other variations of the sequences of Tables II, III and IV, whether such nucleotide changes result in changes in the peptide sequence or not, are also included in the present

invention.

Still a further aspect of the invention is a vector containing a DNA sequence as described above in operative association with an expression control sequence. Such vector may be employed in a novel process for producing a bone growth factor polypeptide in which a cell line transformed with a DNA sequence encoding expression of a bone growth factor polypeptide in operative association with an expression control sequence therefor, is cultured. This claimed process may employ a number of known cells as host cells for expression of the polypeptide. Presently preferred cell lines are mammalian cell lines and bacterial cells.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

Detailed Description of the Invention

The proteins of the present invention are characterized by amino acid sequences or portions thereof the same as or substantially homologous to the sequences shown in Tables II, III and IV. These proteins are also characterized by the ability to induce bone formation.

The bone growth factors provided herein also include factors encoded by the sequences similar to those of Tables II, III and IV, but into which modifications are naturally provided (e.g. allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) or deliberately engineered. For example, synthetic polypeptides may wholly or partially duplicate continuous sequences of the amino acid residues of Tables II, III and IV. These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with bone growth factor polypeptides of Tables II, III and IV may possess bone growth factor biological properties in common therewith. Thus, they may be employed as biologically active substitutes for naturally-occurring bone growth factor polypeptides in therapeutic processes.

Other specific mutations of the sequences of the bone growth factors described herein involve modifications of one or both of the glycosylation sites. The absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at one or both of the asparagine-linked glycosylation recognition sites present in the sequences of the bone growth factors shown in Tables II, III and IV. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for bone growth factors. These DNA sequences include those depicted in Tables II, III and IV in a 5' to 3' direction and those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences of Tables II, III and IV.

DNA sequences which hybridize to the sequences of Tables II, III and IV under relaxed hybridization conditions and which code on expression for bone growth factors having bone growth factor biological properties also encode bone growth factors of the invention. For example, a DNA sequence which shares regions of significant homology, e.g., sites of glycosylation or disulfide linkages, with the sequences of Tables II, III and IV and encodes a bone growth factor having one or more bone growth factor biological properties clearly encodes a member of this novel family of growth factors, even if such a DNA sequence would not stringently hybridize to the sequence of Tables II, III and IV.

Similarly, DNA sequences which code for bone growth factor polypeptides coded for by the sequences of Tables II, III and IV, but which differ in codon sequence due to the degeneracies of the genetic code or allelic variations (naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the novel growth factors described herein. Variations in the DNA sequences of Tables II, III and IV which are caused by point mutations or by induced modifications to enhance the activity, half-life or production of the polypeptides encoded thereby are also encompassed in the invention.

Another aspect of the present invention provides a novel method for producing the novel osteoinductive factors. The method of the present invention involves culturing a suitable cell or cell line, which has been transformed with a DNA sequence coding on expression for a novel bone growth factor polypeptide of the invention, under the control of known regulatory sequences. Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary (CHO) cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293: 620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Another suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. A similarly useful mammalian cell line is the CV-1 cell line.

Bacterial cells are suitable hosts. For example, the various strains of *E. coli* (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas*, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

Another aspect of the present invention provides vectors for use in the method of expression of these novel osteoinductive polypeptides. Preferably the vectors contain the full novel DNA sequences described above which code for the novel factors of the invention. Additionally the vectors also contain appropriate expression control sequences permitting expression of the bone inductive protein sequences. Alternatively, vectors incorporating modified sequences as described above are also embodiments of the present invention and useful in the production of the bone inductive proteins. The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the replication and expression thereof in selected host cells. Useful regulatory sequences for such vectors are known to one of skill in the art and may be selected depending upon the selected host cells. Such selection is routine and does not form part of the present invention.

A protein of the present invention, which induces bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures. An osteogenic preparation employing one or more of the proteins of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. An osteogenic factor of the invention may be valuable in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. Of course, the proteins of the invention may have other therapeutic uses.

A further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to bone defects or periodontal diseases. Such a composition comprises a therapeutically effective amount of at least one of the bone inductive factor proteins of the invention. The bone inductive factors according to the present invention may be present in a therapeutic composition in admixture with a pharmaceutically acceptable vehicle or matrix. Further therapeutic methods and compositions of the invention comprise a therapeutic amount of a bone inductive factor of the invention with a therapeutic amount of at least one of the other bone inductive factors of the invention. Additionally, the proteins according to the present invention or a combination of the proteins of the present invention may be co-administered with one or more different osteoinductive factors with which they may interact. Further, the bone inductive proteins may be combined with other agents beneficial to the treatment of the bone defect in question. Such agents include, but are not limited to various growth factors. The preparation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

In particular, BMP-2 Class I may be used individually in a pharmaceutical composition. BMP-2 Class I may also be used in combination with one or more of the other proteins of the invention. BMP-2 Class I may be combined with BMP-2 Class II. It may also be combined with BMP-3. Further BMP-2 Class I may be combined with BMP-2 Class II and BMP-3.

BMP-2 Class II may be used individually in pharmaceutical composition. In addition, it may be used in combination with other proteins as identified above. Further it may be used in combination with BMP-3.

The therapeutic method includes locally administering the composition as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone damage. Preferably, the bone growth inductive factor composition would include a matrix capable of delivering the bone inductive factor to the site of bone damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of other materials presently in use for other implanted medical applications.

The choice of material is based on, for example, biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. Similarly, the application of the osteoinductive factors will define the appropriate formulation. Potential matrices for the osteoinductive factors may be biodegradable and chemically defined, such as, but not limited to calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyanhydrides; biodegradable and biologically well defined, such as bone or dermal collagen, other pure proteins or extracellular matrix components; nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics; or combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics might also be altered in composition, such as in calcium-aluminate-phos-

phate and processing to alter for example, pore size, particle size, particle shape, and biodegradability.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of such a growth factor, e.g. amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the patient's age, sex, and diet; the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the composition of BMP's. The addition of other known growth factors, such as IGF 1 (insulin like growth factor 1), to the final composition, may also effect the dosage. Generally, the dosage regimen should be in the range of approximately 10 to 10⁶ nanograms of protein per gram of bone weight desired. Progress can be monitored by periodic assessment of bone growth and/or repair, e.g. x-rays. Such therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity in bone inductive factors. Particularly domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with the bone inductive factors of the present invention.

The following examples illustrate practice of the present invention in recovering and characterizing the bovine proteins and employing them to recover the human proteins, obtaining the human proteins and in expressing the proteins via recombinant techniques.

EXAMPLE I

Isolation of Bovine Bone Inductive Factor

Ground bovine bone powder (20-120 mesh, Helitrex) is prepared according to the procedures of M. R. Urist et al., Proc. Natl Acad. Sci USA, 70:3511 (1973) with elimination of some extraction steps as identified below. Ten kgs of the ground powder is demineralized in successive changes of 0.6N HCl at 4°C over a 48 hour period with vigorous stirring. The resulting suspension is extracted for 16 hours at 4°C with 50 liters of 2M CaCl₂ and 10mM ethylenediamine-tetraacetic acid [EDTA], and followed by extraction for 4 hours in 50 liters of 0.5M EDTA. The residue is washed three times with distilled water before its resuspension in 20 liters of 4M guanidine hydrochloride [GuCl], 20mM Tris (pH 7.4), 1 mM N-ethylmaleimide, 1mM iodoacetamide, 1mM phenylmethylsulfonyl fluoride as described in Clin. Orthop. Rel. Res., 171: 213 (1982). After 16 to 20 hours the supernatant is removed and replaced with another 10 liters of GuCl buffer. The residue is extracted for another 24 hours.

The crude GuCl extracts are combined, concentrated approximately 20 times on a Pellicon apparatus with a 10,000 molecular weight cut-off membrane, and then dialyzed in 50mM Tris, 0.1M NaCl, 6M urea (pH7.2), the starting buffer for the first column. After extensive dialysis the protein is loaded on a 4 liter DEAE cellulose column and the unbound fractions are collected.

The unbound fractions are concentrated and dialyzed against 50mM NaAc, 50mM NaCl (pH 4.6) in 6M urea. The unbound fractions are applied to a carboxymethyl cellulose column. Protein not bound to the column is removed by extensive washing with starting buffer, and the bone inductive factor containing material desorbed from the column by 50mM NaAc, 0.25M NaCl, 6M urea (pH 4.6). The protein from this step elution is concentrated 20- to 40- fold, then diluted 5 times with 80mM KPO₄, 6M urea (pH6.0). The pH of the solution is adjusted to 6.0 with 500mM K₂HPO₄. The sample is applied to an hydroxylapatite column (LKB) equilibrated in 80mM KPO₄, 6M urea (pH6.0) and all unbound protein is removed by washing the column with the same buffer. Bone inductive factor activity is eluted with 100mM KPO₄ (pH7.4) and 6M urea.

The protein is concentrated approximately 10 times, and solid NaCl added to a final concentration of 0.15M. This material is applied to a heparin - Sepharose column equilibrated in 50mM KPO₄, 150mM NaCl, 6M urea (pH7.4). After extensive washing of the column with starting buffer, a protein with bone inductive factor activity is eluted by 50mM KPO₄, 700mM NaCl, 6M urea (pH7.4). This fraction is concentrated to a minimum volume, and 0.4ml aliquots are applied to Superose 6 and Superose 12 columns connected in series, equilibrated with 4M GuCl, 20mM Tris (pH7.2) and the columns developed at a flow rate of 0.25ml/min. The protein demonstrating bone inductive factor activity has a relative migration corresponding to approximately 30,000 dalton protein.

The above fractions are pooled, dialyzed against 50mM NaAc, 6M urea (pH4.6), and applied to a Pharmacia MonoS HR column. The column is developed with a gradient to 1.0M NaCl, 50mM NaAc, 6M urea (pH4.6). Active fractions are pooled and brought to pH3.0 with 10% trifluoroacetic acid (TFA). The material is applied to a 0.46 x 25cm Vydac C4 column in 0.1% TFA and the column developed with a gradient to 90% acetonitrile, 0.1% TFA (31.5% acetonitrile, 0.1% TFA to 49.5% acetonitrile, 0.1% TFA in 60 minutes at 1ml per minute). Active material is eluted at approximately 40-44% acetonitrile. Aliquots of the appropriate fractions are iodinated by one of the following methods: P. J. McConahey et al, Int. Arch. Allergy, 29:185-189 (1966); A. E. Bolton et al, Biochem J., 133:529 (1973); and D. F. Bowen-Pope, J. Biol. Chem., 257:5161 (1982). The iodinated proteins present in these fractions are analyzed by SDS gel electrophoresis and urea Triton X 100 isoelectric focusing. At this stage, the bone inductive factor is estimated to be approximately 10-50% pure.

EXAMPLE II

Characterization of Bovine Bone Inductive Factor

A. Molecular Weight

Approximately 20ug protein from Example I is lyophilized and redissolved in 1X SDS sample buffer. After 15 minutes of heating at 37°C, the sample is applied to a 15% SDS polyacrylamide gel and then electrophoresed with cooling. The molecular weight is determined relative to prestained molecular weight standards (Bethesda Research Labs). Immediately after completion, the gel lane containing bone inductive factor is sliced into 0.3cm pieces. Each piece is mashed and 1.4ml of 0.1% SDS is added. The samples are shaken gently overnight at room temperature to elute the protein. Each gel slice is desalted to prevent interference in the biological assay. The supernatant from each sample is acidified to pH 3.0 with 10% TFA, filtered through a 0.45 micron membrane and loaded on a 0.46cm x 5cm C4 Vydac column developed with a gradient of 0.1% TFA to 0.1% TFA, 90% CH₃CN. The appropriate bone inductive factor - containing fractions are pooled and reconstituted with 20mg rat matrix. In this gel system, the majority of bone inductive factor fractions have the mobility of a protein having a molecular weight of approximately 28,000 - 30,000 daltons.

B. Isoelectric Focusing

The isoelectric point of bone inductive factor activity is determined in a denaturing isoelectric focusing system. The Triton X100 urea gel system (Hoeffer Scientific) is modified as follows: 1) 40% of the ampholytes used are Servalyte 3/10; 60% are Servalyte 7-9. 2) The catholyte used is 40mM NaOH. Approximately 20ug of protein from Example I is lyophilized, dissolved in sample buffer and applied to the isoelectrofocusing gel. The gel is run at 20 watts, 10°C for approximately 3 hours. At completion the lane containing bone inductive factor is sliced into 0.5 cm slices. Each piece is mashed in 1.0ml 6M urea, 5mM Tris (pH 7.8) and the samples agitated at room temperature. The samples are acidified, filtered, desalted and assayed as described above. The major portion of activity as determined in the assay described in Example III migrates in a manner consistent with a pI of 8.8 - 9.2.

C. Subunit Characterization

The subunit composition of bone inductive factor is also determined. Pure bone inductive factor is isolated from a preparative 15% SDS gel as described above. A portion of the sample is then reduced with 5mM DTT in sample buffer and re-electrophoresed on a 15% SDS gel. The approximately 30kd protein yields two major bands at approximately 20kd and 18kd, as well as a minor band at 30kd. The broadness of the two bands indicates heterogeneity caused most probably by glycosylation, other post translational modification, proteolytic degradation or carbamylation.

EXAMPLE III

Biological Activity of Bone Inductive Factor

A rat bone formation assay according to the general procedure of Sampath and Reddi, Proc. Natl. Acad. Sci. U. S.A., 80:6591-6595 (1983) is used to evaluate the osteogenic activity of the bovine bone inductive factor of the present invention obtained in Example I. This assay can also be used to evaluate bone inductive factors of other species. The ethanol precipitation step is replaced by dialyzing the fraction to be assayed against water. The solution or suspension is then redissolved in a volatile solvent, e.g. 0.1 - 0.2 % TFA, and the resulting solution added to 20mg of rat matrix. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., Proc. Natl. Acad. Sci., 69:1601 (1972)] and half is fixed and processed for histological analysis. Routinely, 1µm glycolmethacrylate sections are stained with Von Kossa and acid fuchsin to detect new bone mineral. Alkaline phosphatase, an enzyme produced by chondroblasts and osteoblasts in the process of matrix formation, is also measured. New cartilage and bone formation often correlates with alkaline phosphatase levels. Table I below illustrates the dose response of the rat matrix samples including a control not treated with bone inductive factor.

TABLE 1

Protein* Implanted µg	Cartilage	Alk. Phos. u/l
7.5	2	Not done
2.5	3	445.7
0.83	3	77.4
0.28	0	32.5
0.00	0	31.0

*At this stage the bone inductive factor is approximately 10-15% pure.

The bone or cartilage formed is physically confined to the space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing as described above, followed by autoradiography. Analysis reveals a correlation of activity with protein bands at 28 - 30kd and a pI 9.0. An extinction coefficient of 1 OD/mg-cm is used as an estimate for protein and approximating the purity of bone inductive factor in a particular fraction. In the *in vivo* rat bone formation assays on dilutions as described above, the protein is active *in vivo* at 10 to 200ng protein/gram bone to probably greater than 1µg protein/gram bone.

EXAMPLE IV

Bovine Bone Inductive Factor Protein Composition

The protein composition of Example IIA of molecular weight 28 - 30kd is reduced as described in Example IIC and digested with trypsin. Eight tryptic fragments are isolated by standard procedures having the following amino acid sequences:

Fragment 1: A A F L G D I A L D E E D L G

Fragment 2: A F Q V Q Q A A D L

Fragment 3: N Y Q D M V V E G

Fragment 4: S T P A Q D V S R

Fragment 5: N Q E A L R

Fragment 6: L S E P D P S H T L E E

Fragment 7: F D A Y Y

Fragment 8: L K P S N ? A T I Q S I V E

A less highly purified preparation of protein from bovine bone is prepared according to a purification scheme similar to that described in Example I. The purification basically varies from that previously described by omission of the DE-52 column, the CM cellulose column and the mono s column, as well as a reversal in the order of the hydroxylapatite and heparin sepharose columns. Briefly, the concentrated crude 4 M extract is brought to 85% final concentration of ethanol at 4 degrees. The mixture is then centrifuged, and the precipitate redissolved in 50 mM Tris, 0.15 M NaCl, 6.0 M urea. This material is then fractionated on Heparin Sepharose as described. The Heparin bound material is fractionated on hydroxyapatite as described. The active fractions are pooled, concentrated, and fractionated on a high resolution gel filtration (TSK 30000 in 6 M guanidinium chloride, 50 mM Tris, pH 7.2). The active fractions are pooled, dialyzed against 0.1% TFA, and then fractionated on a C4 Vydac reverse phase column as described. The preparation is reduced and electrophoresed on an acrylamide gel. The protein corresponding to the 18K band is eluted and digested with trypsin. Tryptic fragments are isolated having the following amino acid sequences:

Fragment 9: S L K P S N H A T I Q S ? V

Fragment 10: S F D A Y Y C S ? A

Fragment 11: V Y P N M T V E S C A

Fragment 12: V D F A D I ? W

Tryptic Fragments 7 and 8 are noted to be substantially the same as Fragments 10 and 9, respectively.

A. bBMF-2

Two probes consisting of pools of oligonucleotides are designed on the basis of the amino acid sequence of Fragment 3 and synthesized on an automated DNA synthesizer as described above.

Probe #1: A C N A C C A T [A/G] T C [T/C] T G [A/G] A T

Probe #2: C A [A/G] G A [T/C] A T G G T N G T N G A

These probes are radioactively labeled and employed to screen the bovine genomic library constructed as follows: Bovine liver DNA is partially digested with the restriction endonuclease enzyme Sau 3A and sedimented through a sucrose gradient. Size fractionated DNA in the range of 15-30kb is then ligated to the lambda J1 BamH1 arms vector [Frischauf et al, *J. Mol. Biol.*, 170:827-842 (1983) Mullins et al., *Nature* 308: 856-858 (1984)]. The library is plated at 8000 recombinants per plate. Duplicate nitrocellulose replicas of the plaques are made and amplified according to a modification of the procedure of Woo et al, *Proc. Natl. Acad. Sci. USA*, 75:3688-91 (1978).

The radioactively labelled 17-mer Probe #1 is hybridized to the set of filters according to the following method:

The probe is kinased and hybridized to the other set of filters in 3M tetramethylammonium chloride (TMAC), 0.1M sodium phosphate pH6.5, 1mM EDTA, 5X Denhardts, 0.6% SDS, 100ug/ml salmon sperm DNA at 48 degrees C, and washed in 3M TMAC, 50mM Tris pH8.0 at 50 degrees C. These conditions minimize the detection of mismatches to the probe pool [see, Wood et al, *Proc. Natl. Acad. Sci. U.S.A.*, 82:1585-1588 (1985)]. 400,000 recombinants are screened by this procedure. One duplicate positive is plaque purified and the DNA is isolated from a plate lysate of the recombinant bacteriophage designated lambda bP-21. Bacteriophage bP-21 was deposited with the ATCC under accession number ATCC 40310 on March 6, 1987. The bP-21 clone encodes the bovine growth factor designated bBMP-2.

The oligonucleotide hybridizing region of this bBMP-2 clone is localized to an approximately 1.2 kb Sac I restriction fragment which is subcloned into M13 and sequenced by standard techniques. The partial DNA sequence and derived amino acid sequence of this Sac I fragment and the contiguous Hind III-Sac I restriction fragment of bP-21 are shown below in Table II. The bBMP-2 peptide sequence from this clone is 129 amino acids in length and is encoded by the DNA sequence from nucleotide #1 through nucleotide #387. The amino acid sequence corresponding to the tryptic fragment isolated from the bovine bone 28 to 30kd material is underlined in Table II. The underlined portion of the sequence corresponds to tryptic Fragment 3 above from which the oligonucleotide probes for bBMP-2 are designed. The predicted amino acid sequence indicates that tryptic Fragment 3 is preceded by a basic residue (K) as expected considering the specificity of trypsin. The arginine residue encoded by the CGT triplet is presumed to be the carboxy-terminus of the protein based on the presence of a stop codon (TAG) adjacent to it.

TABLE II

5	(1)	15	30	45
	GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG			
	G H D G K G H P L H R R E K R			
10	60	75	90	
	CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG			
	Q A K H K Q R K R L K S S C K			
15	105	120	135	
	AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC			
	R H P L Y V D F S D V G W N D			
20	150	165	180	
	TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG			
	W I V A P P G Y H A F Y C H G			
25	195	210	225	
	GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT			
	E C P F P L A D H L N S T N H			
30	240	255	270	
	GEC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC			
	A I V Q T L V N S V N S K I P			
35	285	300	315	
	AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG			
	K A C C V P T E L S A I S M L			
40	330	345	360	
	TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC			
	Y L D E N E K V V L K N Y Q D			
45	375	(129)	397	407
	ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA			
	M V V E G C G C R			
50	417	427	437	447
	TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC			
55	467	477	487	497
	ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA			
60	517	527	537	547
	AAGAAAAACA CAGCTATTTT GAAAACTATA TTTATATCTA CCGAAAAGAA			
65	567	577	587	
	GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT			

EXAMPLE V**Human Bone Inductive Factors****A. hBMP-2: Class I and II**

The HindIII-SacI bovine genomic bBMP-2 fragment described in Example IV A. is subcloned into an M13 vector. A ³²p-labeled single-stranded DNA probe is made from a template preparation of this subclone. This probe is used to screen polyadenylated RNAs from various cell and tissue sources.

Polyadenylated RNAs from various cell and tissue sources are electrophoresed on formaldehyde-agarose gels and transferred to nitrocellulose by the method of Toole et al., *supra*. The probe is then hybridized to the nitrocellulose blot in 50% formamide, 5 X SSC, 0.1% SDS, 40 mM sodium phosphate pH6.5, 100 µg/ml denatured salmon sperm DNA, and 5 mM vanadyl ribonucleosides at 42° C overnight and washed at 65° C in 0.2 X SSC, 0.1% SDS. Following autoradiography, a hybridizing band corresponding to an mRNA species of approximately 3.8 kb is detected in the lane containing RNA from the human cell line U-2 OS. The HindIII-SacI fragment is labeled with ³²P by nick translation and

used to screen the nitrocellulose filter replicas of a U-2 OS cDNA library by hybridization in standard hybridization buffer at 65° overnight followed by washing in 1 X SSC, 0.1% SDS at 65°.

This library was constructed by synthesizing cDNA from U-2 OS polyadenylated RNA and cloning into lambda gt10 by established techniques (Tool et al., *supra*). Twelve duplicate positive clones are picked and replated for secondaries. Duplicate nitrocellulose replicas are made of the secondary plates and both sets hybridized to the bovine genomic probe as the primary screening was performed. One set of filters is then washed in 1 X SSC, 0.1% SDS; the other in 0.1 X SSC, 0.1% SDS at 65°.

Two classes of hBMP-2 cDNA clones are evident based on strong (4 recombinants) or weak (7 recombinants) hybridization signals under the more stringent washing conditions (0.1 X SSC, 0.1% SDS). All 11 recombinant bacteriophages are plaque purified, small scale DNA preparations made from plate lysates of each, and the inserts subcloned into pSP65 and into M13 for sequence analysis. Sequence analysis of the strongly hybridizing clones designated hBMP-2 Class I (also known as BMP-2) indicates that they have extensive sequence homology with the sequence given in Table II. These clones are therefore cDNA encoding the human equivalent of the protein encoded by the hBMP-2 gene whose partial sequence is given in Table II. Sequence analysis of the weakly hybridizing recombinants designated hBMP-2 Class II (also known as BMP-4) indicates that they are also quite homologous with the sequence given in Table II at the 3' end of their coding regions, but less so in the more 5' regions. Thus they encode a human protein of similar, though not identical, structure to that above.

Full length hBMP-2 Class I cDNA clones are obtained in the following manner. The 1.5 kb insert of one of the Class II subclones (II-10-1) is isolated and radioactively labeled by nick-translation. One set of the nitrocellulose replicas of the U-2 OS cDNA library screened above (50 filters, corresponding to 1,000,000 recombinant bacteriophage) is rehybridized with this probe under stringent conditions (hybridization at 65° in standard hybridization buffer; washing at 65° in 0.2 X SSC, 0.1% SDS). All recombinants which hybridize to the bovine genomic probe which do not hybridize to the Class II probe are picked and plaque purified (10 recombinants). Plate stocks are made and small scale bacteriophage DNA preparations made. After subcloning into M13, sequence analysis indicates that 4 of these represent clones which overlap the original Class I clone. One of these, lambda U2OS-39, contains an approximately 1.5 kb insert and was deposited with the ATCC on June 16, 1987 under accession number 40345. The partial DNA sequence (compiled from lambda U2OS-39 and several other hBMP-2 Class I cDNA recombinants) and derived amino acid sequence are shown below in Table III. Lambda U2OS-39 is expected to contain all of the nucleotide sequence necessary to encode the entire human counterpart of the protein BMP-2 Class I encoded by the bovine gene segment whose partial sequence is presented in Table II. This human cDNA hBMP-2 Class I contains an open reading frame of 1188 bp, encoding a protein of 396 amino acids. This protein of 396 amino acids has a molecular weight of 45kd based on this amino acid sequence. It is contemplated that this sequence represents the primary translation product. The protein is preceded by a 5' untranslated region of 342 bp with stop codons in all frames. The 13 bp region preceding this 5' untranslated region represents a linker used in the cDNA cloning procedure.

TABLE III

5	10	20	30	40	50	60	70
	GTGACTCTA	GAGTGTGT	CAGCACTTG	CTGGGGACTT	CTTGAACCTG	CAGGGAGAAT	AACTTGGCA
10	80	90	100	110	120	130	140
	CCCCACTTIG	GGGGGTGOC	TTTGCCCCAG	CGGAGCCTGC	TTGGCCATCT	CGAGCCCCA	CGCCCCCTCC
15	150	160	170	180	190	200	210
	ACTCTCTGGC	CTTGCCCCAC	ACTGAGAAGC	TGTTCCACAG	GTGAAAAGAG	AGACTGGGOG	GGGGCAACC
20	220	230	240	250	260	270	280
	GGGAGAAGGA	GGAGGCAAG	AAAAGGAAG	GACATTGGT	OCTTGOGCCA	GGTCTTTTGA	CCAGAGTTTT
25	290	300	310	320	330	340	350
	TCCATGTGGA	CGCTCTTTCA	ATGGAAGTGT	CCCCGGTGC	TTCTTAGAAG	GAATGGGTC	TOCTAAAGGT
30	(1)	370	385	400			
	CGACC	ATG GTG GGC GGG ACC GGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC					
		MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val					
35	415	430	445				
	CTC CTG GGC GGC GGC GGT GGC CTC GTT CCG GAG CTG GGC GGC AGG AAG TTC GCG						
	Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala						
40	460	475	490	505			
	GCG GCG TCG TCG GGC GGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG						
	Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu						
45	520	535	550	565			
	TTC GAG TTG GGC CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC						
	Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser						
50	580	595	610				
	AGG GAC GGC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT GGC AGG CAC TCG GGT						
	Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly						
55	625	640	655	670			
	CAG CCG GGC TCA CCC GGC CCA GAC CAC GGC TTG GAG AGG GCA GGC AGC CGA GGC						
	Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala						
	685	700	715				
	AAC ACT GTG GGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG						
	Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr						

730 745 760 775
 AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu
 5 790 805 820 835
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala
 850 865 880
 10 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys
 895 910 925 940
 15 OCT GCA ACA GGC AAC TGG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu
 955 970 985
 20 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET
 1000 1015 1030 1045
 CGG TGG ACT GCA CAG GGA CAC GGC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His
 1060 1075 1090 1105
 25 TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu
 1120 1135 1150
 30 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly
 1165 1180 1195 1210
 35 CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GGC AAA CAC
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His
 1225 1240 1255
 AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp
 1270 1285 1300 1315
 40 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala
 1330 1345 1360 1375
 45 TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
 1390 1405 1420
 50 AAT CAT GGC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT OCT AAG
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys
 55

1435 1450 1465 1480
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu
 5
 1495 1510 1525
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT CCG
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly
 10
 1540(396) 1553 1563 1573 1583 1593 1603
 TGT CCG TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA
 Cys Arg

15
 AAAA

Full-length hBMP-2 Class II human cDNA clones are obtained in the following manner. The 200 bp EcoRI-SacI fragment from the 5' end of the Class II recombinant II-10-1 is isolated from its plasmid subclone, labeled by nick-translation, and hybridized to a set of duplicate nitrocellulose replicas of the U-2 OS cDNA library (25 filters/set; representing 500,000 recombinants). Hybridization and washing are performed under stringent conditions as described above. 16 duplicate positives are picked and replated for secondaries. Nitrocellulose filter replicas of the secondary plates are made and hybridized to an oligonucleotide which was synthesized to correspond to the sequence of II-10-1 and is of the following sequence:

25 **CGGGCGCTCAGGATACTCAAGACCAGTGCTG**

Hybridization is in standard hybridization buffer at 50°C with washing at 50° in 1 X SSC, 0.1% SDS. 14 recombinant bacteriophages which hybridize to this oligonucleotide are plaque purified. Plate stocks are made and small scale bacteriophage DNA preparations made. After subcloning 3 of these into M13, sequence analysis indicates that they represent clones which overlap the original Class II clone. One of these, lambda U2OS-3, was deposited with the ATCC under accession number 40342 on June 16, 1987. U2OS-3 contains an insert of approximately 1.8 kb. The partial DNA sequence and derived amino acid sequence of U2OS-3 are shown below in Table IV. This clone is expected to contain all of the nucleotide sequence necessary to encode the entire human BMP-2 Class II protein. This cDNA contains an open reading frame of 1224 bp, encoding a protein of 408 amino acids, preceded by a 5' untranslated region of 394 bp with stop codons in all frames, and contains a 3' untranslated region of 308 bp following the in-frame stop codon. The 8 bp region preceding the 5' untranslated region represents a linker used in the cDNA cloning procedure. This protein of 408 amino acids has molecular weight of 47kd and is contemplated to represent the primary translation product.

TABLE IV

5	10	20	30	40	50	60	70
	CCTAGAGGG	CAGAGGAGGA	GGGAGGGAGG	GAAGGAGGCG	GGAGCCCGGC	COGGAAGCTA	GGTGAGTGTG
10	80	90	100	110	120	130	140
	GCATCOGAGC	TGAGGGAGCG	GAGCCTGAGA	CGCGCTGCT	GCTOOGGCTG	AGTATCTAGC	TTGTCTCCCC
15	150	160	170	180	190	200	210
	GATGGGATTC	COGTCAAGC	TATCTGAGC	CTGCAGCGCC	ACAGTCCCGG	GOOCTGCGCC	AGGTTCACTG
20	220	230	240	250	260	270	280
	CAACCGTICA	GAGGTCCCCA	GGAGCTGCTG	CTGGGAGGCC	CGCTACTGCA	GGGACCTATG	GAGCATTCOC
25	290	300	310	320	330	340	350
	GTAGTGCCAT	COGAGCAAC	GCACTGCTGC	AGCTTCCCTG	AGCCTTTCCA	GCAAGTTTGT	TCAAGATTGG
30	360	370	380	390	400	(1)	
	CTGTCAAGAA	TCATGGACTG	TTATTATATG	OCTTGTTTTT	TGTCAAGACA	CC ATG ATT OCT	
						MET Ile Pro	
35	417	432	447	462			
	GGT AAC CGA	ATG CTG	ATG GTC	GTT TTA	TTA TGC	CAA GTC	CTG CTA
	Gly Asn Arg	MET Leu	MET Val	Val Leu	Leu Cys	Gln Val	Ileu Leu
40	477	492	507				
	AGC CAT GCT	AGT TTG	ATA OCT	GAG ACG	GGG AAG	AAA AAA	GTC GGC
	Ser His Ala	Ser Leu	Ile Pro	Glu Thr	Gly Lys	Lys Lys	Val Ala
45	522	537	552	567			
	GGC CAC GCG	GGA GGA	CGC CGC	TCA GGG	CAG AGC	CAT GAG	CTC CTG
	Gly His Ala	Gly Gly	Arg Arg	Ser Gly	Gln Ser	His Glu	Leu Leu
50	582	597	612	627			
	GAG GCG ACA	CIT CTG	CAG ATG	TTT GGG	CTG CGC	CGC CGC	COG CAG
	Glu Ala Thr	Leu Leu	Gln MET	Phe Gly	Leu Arg	Arg Arg	Pro Gln
55	642	657	672				
	AGT GGC GTC	ATT CGC	GAC TAC	ATG OGC	GAT CIT	TAC OGC	CIT CAG
	Ser Ala Val	Ile Pro	Asp Tyr	MET Arg	Asp Leu	Tyr Arg	Leu Gln
60	687	702	717	732			
	GAG GAG GAA	GAG CAG	ATC CAQ	AGC ACT	GGT CIT	GAG TAT	OCT GAG
	Glu Glu Glu	Glu Gln	Ile His	Ser Thr	Gly Leu	Glu Tyr	Pro Glu
							Arg Pro

747 762 777
 AGC CGG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile
 5 792 807 822 837
 CCA GGG ACC AGT GAA AAC TCT GCT TTT OGT TTC CTC TTT AAC CTC AGC AGC ATC
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile
 10 852 867 882 897
 OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT OGG CTC TTC OGG GAG CAG GTG
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val
 15 912 927 942
 GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC OGT ATA AAC ATT TAT GAG GTT
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val
 20 957 972 987 1002
 ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp
 1017 1032 1047
 ACG AGA CTG GTC CAC CAC AAT GTG ACA CCG TGG GAA ACT TTT GAT GTG AGC CCT
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro
 25 1062 1077 1092 1107
 GCG GTC CTT OGC TGG ACC OGG GAG AAG CAG CCA AAC TAT GGG CTA GGC ATT GAG
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu
 30 1122 1137 1152 1167
 GIG ACT CAC CTC CAT CAG ACT OGG AOC CAC CAG GGC CAG CAT GTC AGG ATT AGC
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser
 1182 1197 1212
 OGA TOG TTA OCT CAA GGG AGT GGG AAT TGG GOC CAG CTC OGG CCC CTC CTG GTC
 35 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
 1227 1242 1257 1272
 ACC TTT GGC CAT GAT GGC OGG GGC CAT GOC TTG ACC OGA OGC OGG AGG GCC AAG
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Ala Lys
 40 1287 1302 1317
 OGT AGC OCT AAG CAT CAC TCA CAG CCG GOC AGG AAG AAG AAT AAG AAC TGC CCG
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
 1332 1347 1362 1377
 OGC CAC TOG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
 45 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
 1392 1407 1422 1437
 GOC CCA CCA GGC TAC CAG GOC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
 50 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu
 55

1452 1467 1482
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser
 5 1497 1512 1527 1542
 GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
 10 1557 1572 1587
 TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
 1602 1617 (408) 1636 1646 1656
 ATG GTA GTA GAG GGA TGT GGG TGC GCG TGAGATCAGG CAGTCCCTGA GGATAGACAG
 15 MET Val Val Glu Gly Cys Gly Cys Arg
 1666 1676 1686 1696 1706 1716 1726
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CAAGTCCCA TCCACTCAAC CACACACTAC
 20 1736 1746 1756 1766 1776 1786 1796
 ACAGACTGCT TCCCTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC
 25 1806 1816 1826 1836 1846 1856 1866
 ATTCACTTGG ACCCTATTTA TGACTTTTAC TGCAATGTT TTGAACATAT TGATCATATA TTTTGACAAA
 1876 1886 1896 1906 1916 1926 1936
 30 ATATATTTAT AACTACGTAT TAAAAGAAAA AATATAAATG AGTCATTATT TTAAAAAAA AAAAAAACT
 1946
 CTAGAGTGA CGGAATTC

The sequences of BMP-2 Class I and II as shown in Tables II, III IV and have significant homology to the beta (B) and beta (A) subunits of the inhibins. The inhibins are a family of hormones which are presently being investigated for use in contraception. See, A. J. Mason et al, *Nature*, 318:659-663 (1985). To a lesser extent they are also homologous to Mullerian inhibiting substance (MIS), a testicular glycoprotein that causes regression of the Mullerian duct during development of the male embryo and transforming growth factor-beta (TGF-b) which can inhibit or stimulate growth of cells or cause them to differentiate. Furthermore, the sequence of Table IV encoding hBMP-2 Class II has significant homology to the *Drosophila* decapentaplegic (DPP-C) locus transcript. See, J. Massague, *Cell*, 49:437-438 (1987); R. W. Padgett et al, *Nature*, 325:81-84 (1987); R.L. Cate et al, *Cell* 45: 685-698 (1986). It is considered possible therefore that BMP-2 Class II is the human homolog of the protein made from this transcript from this developmental mutant locus.

EXAMPLE VI

Expression of Bone Inductive Factors.

In order to produce bovine, human or other mammalian bone inductive factors, the DNA encoding it is transferred into an appropriate expression vector and introduced into mammalian cells by conventional genetic engineering techniques.

One skilled in the art can construct mammalian expression vectors by employing the sequence of Tables II, III AND IV or other modified sequences and known vectors, such as pCD [Okayama et al., *Mol. Cell Biol.*, 2:161-170 (1982)] and pJL3, pJL4 [Gough et al., *EMBO J.*, 4:645-653 (1985)]. The transformation of these vectors into appropriate host cells can result in expression of osteoinductive factors. One skilled in the art could manipulate the sequences of Tables II, III and IV by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression by bacterial cells. For ex-

ample, the coding sequences could be further manipulated (e.g. ligated to other known linkers or modified by deleting non-coding sequences therefrom or altering nucleotides therein by other known techniques). The modified bone inductive factor coding sequence could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., *Proc. Natl Acad. Sci. USA*, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and bone inductive factor expressed thereby. For a strategy for producing extra-cellular expression of bone inductive factor in bacterial cells., see, e.g. European patent application EPA 177,343.

Similar manipulations can be performed for the construction of an insect vector [see, e.g. procedures described in published European patent application 155,476] for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the present invention by yeast cells. [See, e.g., procedures described in published PCT application W086/00639 and European patent application EPA 123,289].

A method for producing high levels of an osteoinductive factor of the invention from mammalian cells involves the construction of cells containing multiple copies of the heterologous bone inductive factor gene. The heterologous gene can be linked to an amplifiable marker, e.g. the dihydrofolate reductase (DHFR) gene for which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, *J. Mol. Biol.*, 159:601-629 (1982). This approach can be employed with a number of different cell types.

For example, a plasmid containing a DNA sequence for a bone inductive factor of the invention in operative association with other plasmid sequences enabling expression thereof and the DHFR expression plasmid pAdA26SV(A) 3 [Kaufman and Sharp, *Mol. Cell. Biol.*, 2:1304 (1982)] can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate coprecipitation and transfection. DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman et al., *Mol Cell Biol.*, 5: 1750 (1983). Transformants are cloned, and biologically active bone inductive factor expression is monitored by rat bone formation assay. Bone inductive factor expression should increase with increasing levels of MTX resistance. Similar procedures can be followed to produce other bone inductive factors.

Alternatively, the human gene is expressed directly, as described above. Active bone inductive factor may be produced in bacteria or yeast cells. However the presently preferred expression system for biologically active recombinant human bone inductive factor is stably transformed CHO cells.

As one specific example, to produce the human bone inductive factor (hBMP-1) of Example V, the insert of U2OS-1 is released from the vector arms by digestion with Sal I and subcloned into the mammalian expression vector pMT2CX digested with Xho I. Plasmid DNA from this subclone is transfected into COS cells by the DEAE-dextran procedure [Sompayrac and Danna *PNAS* 78:7575-7578 (1981); Luthman and Magnusson, *Nucl.Acids Res.* 11: 1295-1308 (1983)]. Serum-free 24 hr. conditioned medium is collected from the cells starting 40-70 hr. post-transfection.

The mammalian expression vector pMT2 Cla-Xho (pMT2 CX) is a derivative of p91023 (b) (Wong et al., *Science* 228:810-815, 1985) differing from the latter in that it contains the ampicillin resistance gene in place of the tetracycline resistance gene and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 Cla-Xho have been described (Kaufman, R.J., 1985, *Proc. Natl. Acad. Sci. USA* 82:689-693) and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in *E. coli*.

Plasmid pMT2 Cla-Xho is obtained by EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122. EcoRI digestion excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form which can be ligated and used to transform *E. coli* HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods. pMT2CX is then constructed by digesting pMT2 with Eco RV and XbaI, treating the digested DNA with Klenow fragment of DNA polymerase I, and ligating Cla linkers (NEBiolabs, CATCGATG). This removes bases 2266 to 2421 starting from the Hind III site near the SV40 origin of replication and enhancer sequences of pMT2. Plasmid DNA is then digested with EcoRI, blunted as above, and ligated to an EcoRI adapter,

5' PO₄-AATTCCTCGAGAGCT 3'

3' GGAGCTCTCGA 5'

digested with XhoI, and ligated, yielding pMT2 Cla-Xho, which may then be used to transform *E. coli* to ampicillin resistance. Plasmid pMT2 Cla-Xho DNA may be prepared by conventional methods.

Example VIIBiological Activity of Expressed Bone Inductive Factor

5 A. BMP-1

To measure the biological activity of the expressed bone inductive factor, (hBMP-1) obtained in Example VI above. The factor is partially purified on a Heparin Sepharose column. 4 ml of transfection supernatant from one 100 mm dish is concentrated approximately 10 fold by ultrafiltration on a YM 10 membrane and then dialyzed against 20mM Tris, 0.15 M NaCl, pH 7.4 (starting buffer). This material is then applied to a 1.1 ml Heparin Sepharose column in starting buffer. Unbound proteins are removed by an 8 ml wash of starting buffer, and bound proteins, including BMP-1, are desorbed by a 3-4 ml wash of 20 mM Tris, 2.0 M NaCl, pH 7.4.

The proteins bound by the Heparin column are concentrated approximately 10-fold on a Centricon 10 and the salt reduced by diafiltration with 0.1% trifluoroacetic acid. The appropriate amount of this solution is mixed with 20 mg of rat matrix and then assayed for *in vivo* bone and cartilage formation as previously described in Example III. A mock transfection supernatant fractionation is used as a control.

The implants containing rat matrix to which specific amounts of human BMP-1 have been added are removed from rats after seven days and processed for histological evaluation. Representative sections from each implant are stained for the presence of new bone mineral with von Kossa and acid fuchsin, and for the presence of cartilage-specific matrix formation using toluidine blue. The types of cells present within the section, as well as the extent to which these cells display phenotype are evaluated.

Addition of human BMP-1 to the matrix material resulted in formation of cartilage-like nodules at 7 days post implantation. The chondroblast-type cells were recognizable by shape and expression of metachromatic matrix. The amount of activity observed for human BMP-1 was dependent upon the amount of human BMP-1 protein added to the matrix. Table IX illustrates the dose-response relationship of human BMP-1 protein to the amount of bone induction observed.

Table IX

IMPLANT NUMBER	AMOUNT USED (equivalent of ml transfection media)	HISTOLOGICAL SCORE
876-134-1	10 BMP-1	C+2
876-134-2	3 BMP-1	C+1
876-134-3	1 BMP-1	C+/-
876-134-4	10 MOCK	C-
876-134-5	3 MOCK	C -
876-134-6	1 MOCK	C -

Cartilage (c) activity was scored on a scale from 0(-) to 5.

Similar levels of activity are seen in the Heparin Sepharose fractionated COS cell extracts. Partial purification is accomplished in a similar manner as described above except that 6 M urea is included in all the buffers. Further, in a rat bone formation assay as described above, BMP-2 has similarly demonstrated chondrogenic activity.

The procedures described above may be employed to isolate other bone inductive factors of interest by utilizing the bovine bone inductive factors and/or human bone inductive factors as a probe source. Such other bone inductive factors may find similar utility in, inter alia, fracture repair.

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto.

50 **Claims.**

Claims for the following Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A gene encoding human BMP-2 comprising the following DNA sequence:

10 20 30 40 50 60 70
 GTGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACCTG CAGGGAGAAT AACTTGGCA
 5
 80 90 100 110 120 130 140
 CCCCACCTTG CGCGGGTGOC TTGTGCCCCAG CGGAGCCTGC TTGGCCATCT CCGAGCCCCA CCGCCCCCTCC
 10
 150 160 170 180 190 200 210
 ACTCTCGGC CTGTGCCCCAC ACTGAGAGGC TGTTCGCCAG GTGAAAAGAG AGACTGGGCG GCGGGCACCC
 15
 220 230 240 250 260 270 280
 GGGAGAAGGA GGAGGCAAG AAAAGGAACG GACATTGGT CCTTGGGCA GGTCTTTTGA CCAGAGTTTT
 290 300 310 320 330 340 350
 TCATGTGGA CGCTCTTTCA ATGGAAGTGT CCGCGGTGC TTCTAGAGC GACTGGGGTC TCTAAAGGT
 20
 (1) 370 385 400
 CGACC ATG GTG GGC AOC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val
 25
 415 430 445
 CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GCG
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala
 30
 460 475 490 505
 GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu
 35
 520 535 550 565
 TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser
 40
 580 595 610
 AGG GAC GGC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CGC AGG CAC TCG GGT
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly
 45
 625 640 655 670
 CAG CCG GGC TCA CCC GGC CCA GAC CAC CGG TTG GAG AGG GCA GCC AGC CGA GCC
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala
 50
 685 700 715
 AAC ACT GTG CGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr
 55

730 745 760 775
 AGT GGG AAA ACA ACC OGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu
 5
 790 805 820 835
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala
 10
 850 865 880
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys
 15
 895 910 925 940
 OCT GCA ACA GOC AAC TOG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu
 20
 955 970 985
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET
 25
 1000 1015 1030 1045
 OGG TGG ACT GCA CAG GGA CAC GOC AAC CAT GGA TTC GTG GTG GAA GTG GOC CAC
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His
 30
 1060 1075 1090 1105
 TTG GAG GAG AAA CAA GGT GTC TOC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu
 35
 1120 1135 1150
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly
 40
 1165 1180 1195 1210
 CAT GAT GGA AAA GGG CAT OCT CTC CAC AAA AGA GAA AAA CGT CAA GOC AAA CAC
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His
 45
 1225 1240 1255
 AAA CAG OGG AAA OGC CTT AAG TOC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp
 50
 1270 1285 1300 1315
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GGC
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala
 55
 1330 1345 1360 1375
 TTT TAC TGC CAC GGA GAA TGC OCT TTT OCT CTG GCT GAT CAT CTG AAC TOC ACT
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
 1390 1405 1420
 AAT CAT GOC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT OCT AAG
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

1435 1450 1465 1480
GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu
5
1495 1510 1525
AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GCG
Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly
10
1540(396) 1553 1563 1573 1583 1593 1603
TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA
Cys Arg

15 AAAAA

2. A gene encoding human BMP-2 having the amino acid sequence given in claim 1.
3. A gene encoding a protein exhibiting properties of human BMP-2 and comprising a DNA sequence:
 - (a) which differs from a DNA sequence of claim 1 in codon sequence due to the degeneracy of the genetic code;
 - (b) which hybridises with a DNA sequence of claim 1 or section (a), above; or
 - (c) represents a fragment, allelic or other variation of a DNA sequence of claim 1, whether said variation results in changes in the peptide sequence or not.
4. The DNA sequence of claim 3, which is a genomic DNA sequence.
5. The DNA sequence of claim 3, which is a cDNA sequence.
6. A gene encoding bovine BMP-2 comprising the following DNA sequence:

(1) 15 30 45
GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
G H D G K G H P L H R R E K R

5 60 75 90
CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG
Q A K H K Q R K R L K S S C K

10 105 120 135
AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC
R H P L Y V D F S D V G W N D

15 150 165 180
TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG
W I V A P P G Y H A F Y C H G

20 195 210 225
GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
E C P F P L A D H L N S T N H

25 240 255 270
GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC
A I V Q T L V N S V N S K I P

30 385 300 315
AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG
K A C C V P T E L S A I S M L

35 330 345 360
TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC
Y L D E N E K V V L K N Y O D

40 375 (129) 397 407
ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA
M V V E G C G C R

45 417 427 437 447 457
TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC

50 467 477 487 497 507
ACTTTAATAT TTCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA

55 517 527 537 547 557
AAGAAAAACA CAGCTATTTT GAAAACATA TTTATATCTA CCGAAAAGAA

567 577 587
GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT

7. A gene encoding bovine BMP-2 containing the amino acid sequence of claim 6.

8. A gene encoding a protein exhibiting properties of bovine BMP-2 and comprising DNA sequences:

- (a) which differ from a DNA sequence of claim 7 in codon sequence due to the degeneracy of the genetic code;
(b) which hybridise with a DNA sequence of claim 7 or section (a), above; or
(c) represent fragments, allelic or other variations of a DNA sequence of claim 7, whether said variations result in changes in the peptide sequence or not.

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9. The DNA sequence of claim 8, which is a genomic DNA sequence.

10. The DNA sequence of claim 8, which is a cDNA sequence.

5 11. A gene encoding human BMP-4 comprising the following DNA sequence :

```

10      10      20      30      40      50      60      70
CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGGCG GGAGCCCGGC CCGGAAGCTA GGTGAGTGTG

      80      90     100     110     120     130     140
GCATCCGAGC TGAGGGAAGC GAGCCTGAGA CGCGCTGCTT GCTCCCGCTG AGTATCTAGC TTGTCTCCCC

15      150     160     170     180     190     200     210
GATGGGATTC CCGTCCAAGC TATCTGAGC CTGCAGGCGC ACAGTCCCGG GGCCTGGGCG AGGTTCACCTG

      220     230     240     250     260     270     280
20 CAACCGTTCA GAGGTCCCA GAGCTGCTG CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCATTCCT

      290     300     310     320     330     340     350
25 GTAGTGGCAT CCGAGCAAC GCACCTGCTG AGCTCCCTG AGCCTTCCA GCAAGTTTGT TCAAGATGG

      360     370     380     390     400     (1)
30 CTGTCAAGAA TCATGGACTG TTATTATATG CCTTGTGTTT TGTCAAGACA CC ATG ATT OCT
MET Ile Pro

```


417 432 447 462
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GGG
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala
 5
 477 492 507
 AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GGC GAG ATT CAG
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln
 10
 522 537 552 567
 GGC CAC GCG GGA GGA GGC GGC TCA GGG CAG AGC CAT GAG CTC CTG CCG GAC TTC
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe
 15
 582 597 612 627
 GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG GGC GGC GGC CCG CAG CCT AGC AAG
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys
 20
 642 657 672
 AGT GGC GTC ATT CCG GAC TAC ATG GGC GAT CTT TAC GGC CTT CAG TCT GGG GAG
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu
 25
 687 702 717 732
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG GGC CCG GGC
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala
 30
 747 762 777
 AGC GGC GGC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile
 35
 792 807 822 837
 CCA GGC ACC AGT GAA AAC TCT GCT TTT GGT TTC CTC TTT AAC CTC AGC AGC ATC
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile
 40
 852 867 882 897
 OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT GGC CTC TTC CCG GAG CAG GTG
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val
 45
 912 927 942
 GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC GGT ATA AAC ATT TAT GAG GTT
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val
 50
 957 972 987 1002
 ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp
 55
 1017 1032 1047
 ACG AGA CTG GTC CAC CAC AAT GTG ACA GGC TGG GAA ACT TTT GAT GTG AGC OCT
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro
 1062 1077 1092 1107
 GCG GTC CTT GGC TGG ACC GGC GAG AAG CAG CCA AAC TAT GGC CTA GGC ATT GAG
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu
 1122 1137 1152 1167
 GTG ACT CAC CTC CAT CAG ACT GGC ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

1182 1197 1212
 CGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GGC CAG CTC OGG CCC CTC CTG GTC
 5 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
 1227 1242 1257 1272
 ACC TTT GGC CAT GAT GGC OGG GGC CAT GCC TTG ACC CGA CGC OGG AGG GCC AAG
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys
 10 1287 1302 1317
 OGT AGC OCT AAG CAT CAC TCA CAG OGG GGC AGG AAG AAG AAT AAG AAC TGC OGG
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
 1332 1347 1362 1377
 15 OGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
 1392 1407 1422 1437
 20 GCC CCA CCA GGC TAC CAG GGC TTC TAC TGC CAT GGG GAC TGC CCC TTT OCA CTG
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asn Cys Pro Phe Pro Leu
 1452 1467 1482
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser
 25 1497 1512 1527 1542
 GTC AAT TCC AGT ATC CCC AAA GGC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
 1557 1572 1587
 30 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
 1602 1617 (408) 1636 1646 1656
 35 ATG GTA GTA GAG GGA TGT GGG TGC OGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
 MET Val Val Glu Gly Cys Gly Cys Arg
 1666 1676 1686 1696 1706 1716 1726
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCAAC CACACACTAC
 40 1736 1746 1756 1766 1776 1786 1796
 ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAATAA AATGGAAAAA ATCCTAAAC
 1806 1816 1826 1836 1846 1856 1866
 45 ATTCAACCTTG ACCCTATTTA TGACTTTACG TGCAAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA
 1876 1886 1896 1906 1916 1926 1936
 50 ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTTAAAAAAA AAAAAAACT
 1946
 CTAGAGTCGA CGGAATTC
 55

12. A gene encoding human BMP-4 having the amino acid sequence given in claim 11.

13. A gene encoding a protein exhibiting properties of BMP-4 and comprising a DNA sequence:

- (a) which differs from a DNA sequence of claim 11 in codon sequence due to the degeneracy of the genetic code;
 (b) which hybridises with a DNA sequence of claim 11 or section (a), above; or
 (c) represents a fragment, allelic or other variation of a DNA sequence of claim 11, whether said variation results in changes in the peptide sequence or not.

14. The DNA sequence of claim 13, which is a genomic DNA sequence.

15. The DNA sequence of claim 13, which is a cDNA sequence.

16. A vector containing the gene or DNA sequence of any one of claims 1 to 15 in operative association with an expression control sequence.

17. A cell transformed with a vector of claim 16.

18. The cell of claim 17 which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.

19. The cell of claim 18 which is a CHO cell.

20. A protein exhibiting properties of BMP-2 which is encoded by a gene or DNA sequence of any one of claims 1 to 10.

21. A protein exhibiting properties of BMP-2, which is obtainable by the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence of any one of claims 1 to 10, and recovering said protein from said culture medium.

22. A protein exhibiting properties of BMP-4 which is encoded by a gene or DNA sequence of any one of claims 11 to 15.

23. A protein exhibiting properties of BMP-4, which is obtainable by the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence of any one of claims 11 to 15, and recovering said protein from said culture medium.

24. A process for producing the protein of claims 21 or 23, comprising the steps of culturing in a suitable culture medium the cell of claim 17 and isolating said protein from said culture medium.

25. A pharmaceutical composition comprising the proteins of any one of claims 20 to 23, individually or in combination, and a pharmaceutically acceptable vehicle.

26. The pharmaceutical composition of claim 25, further comprising a matrix capable of delivering the composition to the site of the bone or cartilage defect and providing a structure for inducing bone or cartilage formation.

27. The pharmaceutical composition of claim 26, wherein said matrix comprises hydroxyapatite, collagen, polylactic acid or tricalcium phosphate.

28. Use of a protein of any one of claims 20 to 23, individually or in combination, for the preparation of a pharmaceutical composition for inducing bone or cartilage formation.

Claims for the following Contracting State : AT

1. A process for the preparation of a gene encoding human BMP-2 comprising the following DNA sequence:

10 20 30 40 50 60 70
 GTGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTGGAACITG CAGGGAGAAT AACTTGCGCA
 5
 80 90 100 110 120 130 140
 CCGCACTTTG CGGCGGTGOC TTTGCCCCAG CCGAGCGCTC TTGCGCATCT CCGAGCCCCA CCGCCCCCTCC
 10
 150 160 170 180 190 200 210
 ACTOCTGGC CTTGCCCCGAC ACTGAGAAGC TGTTCOCAGC GTGAAAAGAG AGACTGCGCG GCGGCGACCC
 15
 220 230 240 250 260 270 280
 GGGAGAAGCA GGAGGCAAAG AAAAGGAAAG GACATTGGT CTTGCGGCA GGTCTTTTGA CCAGAGTTTT
 20
 290 300 310 320 330 340 350
 TCCATGTGGA CGCTCTTTCA ATGGAAGTGT CCGCGGTGTC TCTTAGAGC GACTCGGGTC TCCTAAGGT
 (1) 370 385 400
 CGACC ATG GTG GGC GGG ACC GGC TGT CTT CTA GCG TTG CTG CTT CCG CAG GTC
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val
 25
 415 430 445
 CTC CTG GGC GGC GCG GGT GGC CTC GTT CCG GAG CTG GGC GGC AGG AAG TTC GCG
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala
 30
 460 475 490 505
 GCG GCG TOG TOG GGC GGC CCG TCA TCC CAG CCG TCT GAC GAG GTC CTG AGC GAG
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu
 35
 520 535 550 565
 TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCG ACC CCG AGC
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser
 580 595 610
 AGG GAC GGC GTG GTG CCG CCG TAC ATG CTA GAC CTG TAT CCG AGG CAC TOG GGT
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly
 40
 625 640 655 670
 CAG CCG GGC TCA CCG GGC CCA GAC CAC CCG TTG GAG AGG GCA GCG AGC CGA GCG
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala
 45
 685 700 715
 AAC ACT GTG CCG AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA AGC
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr
 50
 55

730 745 760 775
 AGT GGG AAA ACA ACC OGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu
 5
 790 805 820 835
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala
 10
 850 865 880
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys
 15
 895 910 925 940
 OCT GCA ACA GGC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu
 20
 955 970 985
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET
 25
 1000 1015 1030 1045
 OGG TGG ACT GCA CAG GGA CAC GGC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His
 30
 1060 1075 1090 1105
 TTG GAG GAG AAA CAA GGT GTC TOC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu
 35
 1120 1135 1150
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG OCA TTG CTA GTA ACT TTT GGC
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly
 40
 1165 1180 1195 1210
 CAT GAT GGA AAA GGG CAT OCT CTC CAC AAA AGA GAA AAA OGT CAA GGC AAA CAC
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His
 45
 1225 1240 1255
 AAA CAG OGG AAA OGC CTT AAG TOC AGC TGT AAG AGA CAC OCT TTG TAC GTG GAC
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp
 50
 1270 1285 1300 1315
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC OCG GGG TAT CAC GCC
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala
 55
 1330 1345 1360 1375
 TTT TAC TGC CAC GGA GAA TGC OCT TTT OCT CTG GCT GAT CAT CTG AAC TOC ACT
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
 1390 1405 1420
 AAT CAT GGC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT OCT AAG
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

1435 1450 1465 1480
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
 5 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu

 1495 1510 1525
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG
 10 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly

 1540(396) 1553 1563 1573 1583 1593 1603
 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA
 Cys Arg

15 AAAA ,

wherein said process comprises the following steps:

- 20 a) screening of a gene library constructed from U-2 OS derived DNA or cDNA with a labelled bBMP-2 fragment by hybridization,

 b) isolating positive clones, and

 c) isolating the DNA-inserts from said clones.
- 25 2. The process according to claim 1, wherein the gene encodes human BMP-2 having the amino acid sequence given in claim 1.
- 30 3. A process for the preparation of a gene encoding a protein exhibiting properties of human BMP-2 and comprising a DNA sequence:

 - a) which differs from a DNA sequence of claim 1 in codon sequence due to the degeneracy of the genetic code;
 - b) which hybridizes with a DNA sequence of claim 1 or section (a), above; or
 - c) represents a fragment, allelic or other variation of a DNA sequence of claim 1, whether said variation results in changes in the peptide sequence or not,
- 35 wherein said process comprises standard techniques of molecular biology.
4. The process according to claim 3, wherein the DNA sequence is a genomic DNA sequence.
- 40 5. The process according to claim 3, wherein the DNA sequence is a cDNA sequence.
- 55 6. A process for the preparation of a gene encoding bovine BMP-2 comprising the following DNA sequence:

(1) 15 30 45
GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
G H D G K G H P L H R R E K R

5

60 75 90
CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG
Q A K H K Q R K R L K S S C K

10

105 120 135
AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC
R H P L Y V D F S D V G W N D

15

150 165 180
TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG
W I V A P P G Y H A F Y C H G

20

195 210 225
GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
E C P F P L A D H L N S T N H

25

240 255 270
GEC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC
A I V Q T L V N S V N S K I P

30

385 300 315
AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG
K A C C V P T E L S A I S M L

330 345 360
TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC
Y L D E N E K V V L K N Y Q D

35

375 (129) 397 407
ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA
M V V E G C G C R

40

417 427 437 447 457
TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC

467 477 487 497 507
ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA

45

517 527 537 547 557
AAGAAAAACA CAGCTATTTT GAAACTATA TTTATATCTA CCGAAAAGAA

567 577 587
GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT,

wherein said process comprises the following steps:

- a) screening a gene library constructed from bovine liver DNA or cDNA with a labelled probe designed on the basis of the amino acid sequence of a fragment of bBMP-2,
- b) isolating positive clones, and
- c) isolating the DNA-inserts from said clones.

7. The process according to claim 6, wherein the gene encodes bovine BMP-2 having the amino acid sequence of claim 6.

8. A process for the preparation of a gene encoding a protein exhibiting properties of bovine BMP-2 and comprising DNA sequences:

5 a) which differ from a DNA sequence of claim 7 in codon sequence due to the degeneracy of the genetic code;
 b) which hybridize with a DNA sequence of claim 7 or section a), above; or
 c) represent fragments, allelic or other variations of a DNA sequence of claim 7, whether said variations result in changes in the peptide sequence or not,

10 wherein said process comprises standard techniques of molecular biology.

9. The process according to claim 8, wherein the DNA sequence is a genomic DNA sequence.

10. The process according to claim 8, wherein the DNA sequence is a cDNA sequence.

- 15 11. A process for the preparation of a gene encoding human BMP-4 comprising the following DNA sequence:

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747 762 777
 AGC OGG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile
 5
 792 807 822 837
 OCA GGG ACC AGT GAA AAC TCT GCT TTT OGT TTC CTC TTT AAC CTC AGC AGC ATC
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile
 10
 852 867 882 897
 OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT OGG CTC TTC OGG GAG CAG GTG
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val
 15
 912 927 942
 GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC OGT ATA AAC ATT TAT GAG GTT
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val
 20
 957 972 987 1002
 ATG AAG CCC OCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA OGA CTA CTG GAC
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp
 25
 1017 1032 1047
 ACG AGA CTG GTC CAC CAC AAT GTG ACA OGG TGG GAA ACT TTT GAT GTG AGC OCT
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro
 30
 1062 1077 1092 1107
 GOG GTC CTT OGC TGG ACC OGG GAG AAG CAG OCA AAC TAT GGG CTA GCC ATT GAG
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu
 35
 1122 1137 1152 1167
 GTG ACT CAC CTC CAT CAG ACT OGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser
 40
 1182 1197 1212
 OGA TGG TTA OCT CAA GGG AGT GGG AAT TGG GCC CAG CTC OGG CCC CTC CTG GTC
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
 45
 1227 1242 1257 1272
 ACC TTT GGC CAT GAT GGC OGG GGC CAT GCC TTG ACC OGA OGC OGG AGG GCC AAG
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Ala Lys
 50
 1287 1302 1317
 OGT AGC OCT AAG CAT CAC TCA CAG OGG GCC AGG AAG AAG AAT AAG AAC TGC OGG
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
 55
 1332 1347 1362 1377
 OGC CAC TGG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
 1392 1407 1422 1437
 GCC OCA OCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT OCA CTG
 Ala Pro Pro Gly Trp Gln Ala Phe Trp Cys His Glv Asp Cys Pro Phe Pro Leu
 1452 1467 1482
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

1497 1512 1527 1542
 GTC AAT TOC AGT ATC CCC AAA GGC TGT TGT GTG CCC ACT GAA CTG AGT GGC ATC
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
 5
 1557 1572 1587
 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
 10
 1602 1617 (408) 1636 1646 1656
 ATG GTA GTA GAG GGA TGT GGG TGC GGC TGAGATCAGG CAGTCCTGA GGATAGACAG
 MET Val Val Glu Gly Cys Gly Cys Arg
 1666 1676 1686 1696 1706 1716 1726
 15 ATATACACAC CACACACACA CACCACATAC ACCACACACA CAOGTTCCCA TOCACTCAAC CACACACTAC
 1736 1746 1756 1766 1776 1786 1796
 20 ACAGACTGCT TOCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCOCTAAAC
 1806 1816 1826 1836 1846 1856 1866
 ATTCAOCTTG AOCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA
 25
 1876 1886 1896 1906 1916 1926 1936
 ATATATTTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAA AAAAAAACT
 30
 1946
 CTAGAGTCGA CGGAATTC ,

wherein said process comprises the following steps:

- 35 a) screening of a gene library constructed from U-2 OS derived DNA or cDNA with a labelled bBMP-2 fragment by hybridization,
 b) isolating positive clones, and
 c) isolating the DNA-inserts from said clones.
- 40 12. The process according to claim 11, wherein the gene encodes human BMP-4 having the amino acid sequence given in claim 11.
- 45 13. A process for the preparation of a gene encoding a protein exhibiting properties of BMP-4 and comprising a DNA sequence:
 a) which differs from a DNA sequence of claim 11 in codon sequence due to the degeneracy of the genetic code;
 b) which hybridizes with DNA sequence of claim 11 or section a), above; or
 c) represents a fragment, allelic or other variation of a DNA sequence of claim 11, whether said variation results in changes in the peptide sequence or not,
 50
 wherein said process comprises standard techniques of molecular biology.
14. The process according to claim 13, wherein the DNA sequence is a genomic DNA sequence.
- 55 15. The process according to claim 13, wherein the DNA sequence is a cDNA sequence.
16. A vector containing the gene or DNA sequence prepared according to any one of claims 1 to 15 in operative association with an expression control sequence.

17. A cell transformed with a vector of claim 16.

18. The cell of claim 17 which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.

5 19. The cell of claim 18 which is a CHO cell.

10 20. A process for the preparation of a protein exhibiting properties of BMP-2, wherein said process comprises the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence prepared according to any one of claims 1 to 10, and recovering said protein from said culture medium.

15 21. A process for the preparation of a protein exhibiting properties of BMP-4, wherein said process comprises the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence prepared according to any one of claims 11 to 15, and recovering said protein from said culture medium.

22. A process for producing a protein exhibiting properties of BMP-2 or BMP-4, comprising the steps of culturing in a suitable culture medium the cell of claim 17 and isolating said protein from said culture medium.

20 23. A process for the preparation of a pharmaceutical composition comprising combining the proteins prepared according to any one of claims 20 to 22, individually or in combination with a pharmaceutically acceptable vehicle.

25 24. The process according to claim 23, wherein said pharmaceutical composition further comprises a matrix capable of delivering the composition to the site of the bone or cartilage defect and providing a structure for inducing bone or cartilage formation.

25 25. The process according to claim 24, wherein said matrix comprises hydroxyapatite, collagen, polylactic acid or tricalcium phosphate.

30 26. Use of a protein prepared according to any one of claims 20 to 22, individually or in combination, for the preparation of a pharmaceutical composition for inducing bone or cartilage formation.

35 Patentansprüche

Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

40 1. Menschliches BMP-2 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

45

50

55

10 20 30 40 50
 GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG
 5 60 70 80 90 100
 CAGGGAGAAT AACTTGCGCA CCCCACTTTG CGCCGGTGCC TTTGCCCCAG
 110 120 130 140 150
 CGGAGCCTGC TTCGCCATCT CCGAGCCCCA CCGCCCCTCC ACTCCTCGGC
 10 160 170 180 190 200
 CTTGCCCCGAC ACTGAGACGC TGTTCCCGAGC GTGAAAAGAG AGACTGCGCG
 210 220 230 240 250
 15 GCCGGCACCC GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTCGGT
 260 270 280 290 300
 CCTTGCGCCA GGTCCCTTTGA CCAGAGTTTT TCCATGTGGA CGCTCTTTCA
 20 310 320 330 340 350
 ATGGACGTGT CCCC GCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT
 (1) 370 385 400
 25 CGAOC ATG GTG GOC GGG AOC CGC TGT CTT CTA GOG TTG CTG CTT OCC CAG GTC
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val
 415 430 445
 30 CTC CTG GGC GGC GOG GCT GGC CTC GTT OCG GAG CTG GGC OGC AGG AAG TTC GOG
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala
 460 475 490 505
 GOG GOG TOG TOG GGC OGC OCC TCA TOC CAG OCC TCT GAC GAG GTC CTG AGC GAG
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu
 35 520 535 550 565
 TTC GAG TTG OGG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA OCC AOC OCC AGC
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser
 40 580 595 610
 AGG GAC GOC GTG GTG OCC OCC TAC ATG CTA GAC CTG TAT OGC AGG CAC TOG GGT
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly
 45
 50
 55

625 640 655 670
 CAG CCG GGC TCA CCG GGC CCA GAC CAC CCG TTG GAG AGG GCA GGC AGC CGA GGC
 5 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala

685 700 715
 AAC ACT GTG CCG AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG
 10 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr

730 745 760 775
 AGT GGG AAA ACA AOC CCG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCG ACG GAG
 15 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu

790 805 820 835
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala

850 865 880
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
 20 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys

895 910 925 940
 OCT GCA ACA GGC AAC TCG AAA TTC CCG GTG ACC AGT CTT TTG GAC ACC AGG TTG
 25 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu

955 970 985
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCG GCT GTG ATG
 30 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET

1000 1015 1030 1045
 CCG TGG ACT GCA CAG GGA CAC GGC AAC CAT GGA TTC GTG GTG GAA GTG GGC CAC
 35 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His

1060 1075 1090 1105
 TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
 40 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu

1120 1135 1150
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly

1165 1180 1195 1210
 CAT GAT GGA AAA GGG CAT CTT CTC CAC AAA AGA GAA AAA CGT CAA GGC AAA CAC
 45 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His

1225 1240 1255
 AAA CAG CCG AAA CCG CTT AAG TCC AGC TGT AAG AGA CAC OCT TTG TAC GTG GAC
 50 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp

1270 1285 1300 1315
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCG CCG GGG TAT CAC GGC
 55 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala

1330 1345 1360 1375
 TTT TAC TGC CAC GGA GAA TGC OCT TTT OCT CTG GCT GAT CAT CTG AAC TCC ACT
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
 5
 1390 1405 1420
 AAT CAT GGC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT OCT AAG
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys
 10
 1435 1450 1465 1480
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu
 15
 1495 1510 1525
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GCG
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly
 20
 1540(396) 1553 1563 1573 1583 1593 1603
 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAC AAAAAAGAAA
 Cys Arg
 25
 AAAA

2. Gen, das menschliches BMP-2 codiert, das die in Anspruch 1 angegebene Aminosäuresequenz aufweist.
3. Gen, das ein Protein codiert, das Eigenschaften von menschlichem BMP-2 zeigt, und eine DNA-Sequenz umfaßt, die:
 - (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 1 unterscheidet;
 - (b) mit einer DNA-Sequenz nach Anspruch 1 oder nach vorstehendem Absatz (a) hybridisiert; oder
 - (c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 1 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht.
4. DNA-Sequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.
5. DNA-Sequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.
6. Rinder-BMP-2 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

(1) 15 30 45
 GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
 G H D G K G H P L H R R E K R
 5
 60 75 90
 CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG
 Q A K H K Q R K R L K S S C K
 10
 105 120 135
 AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC
 R H P L Y V D F S D V G W N D
 15
 150 165 180
 TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG
 W I V A P P G Y H A F Y C H G
 20
 195 210 225
 GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
 E C P F P L A D H L N S T N H
 25
 240 255 270
 GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC
 A I V Q T L V N S V N S K I P
 30
 385 300 315
 AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG
 K A C C V P T E L S A I S M L
 35
 330 345 360
 TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC
 Y L D E N E K V V L K N Y Q D
 40
 375 (129) 397 407
 ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA
 M V V E G C G C R
 45
 417 427 437 447 457
 TAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC
 467 477 487 497 507
 ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA
 517 527 537 547 557
 AAGAAAAACA CAGCTATTTT GAAACTATA TTTATATCTA CCGAAAAAGAA
 567 577 587
 GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT ,
 50

7. Gen, das Rinder-BMP-2 codiert, das die Aminosäuresequenz von Anspruch 6 enthält.

8. Gen, das ein Protein codiert, das Eigenschaften von Rinder-BMP-2 zeigt, und DNA-Sequenzen umfaßt, die:

(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 7 unterscheiden;

(b) mit einer DNA-Sequenz nach Anspruch 7 oder nach vorstehendem Absatz (a) hybridisieren; oder

(c) Fragmente, allelisch oder andere Variationen einer DNA-Sequenz nach Anspruch 7 darstellen, unabhängig davon, ob die Variationen zu Änderungen in der Peptidsequenz führen oder nicht.

5

9. DNA-Sequenz nach Anspruch 8, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.

10. DNA-Sequenz nach Anspruch 8, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.

10

11. Menschliches BMP-4 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

	10	20	30	40	50
	CTCTAGAGGG	CAGAGGAGGA	GGGAGGGAGG	GAAGGAGCGC	GGAGCCCCGGC

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10
15
20
25

60 70 80 90 100
CCGGAAGCTA GGTGAGTGTG GCATCCGAGC TGAGGGACGC GAGCCTGAGA

110 120 130 140 150
CGCCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCC GATGGGATTC

160 170 180 190 200
CCGTCCAAGC TATCTCGAGC CTGCAGCGCC ACAGTCCCCG GCCCTCGCCC

210 220 230 240 250
AGGTTCACTG CAACCGTTCA GAGGTCCCCA GGAGCTGCTG CTGGCGAGCC

260 270 280 290 300
CGCTACTGCA GGGACCTATG GAGCCATTCC GTAGTGCCAT CCCGAGCAAC

310 320 330 340 350
GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG

360 370 380 390 400
CTGTCAAGAA TCATGGACTG TTATTATATG CCTTGTTTTT TGTCAAGACA

(1)
CC ATG ATT CCT
MET Ile Pro

30
35
40
45
50
55

417 432 447 462
GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG
Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala

477 492 507
AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG
Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln

522 537 552 567
GGC CAC GCG GGA GGA GCG GCG TCA GGG CAG AGC CAT GAG CTC CTG CCG GAC TTC
Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe

582 597 612 627
GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG GCG GCG GCG CAG OCT AGC AAG
Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys

642 657 672
AGT GCG GTC ATT CCG GAC TAC ATG CCG GAT CTT TAC CCG CTT CAG TCT GGG GAG
Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu

687 702 717 732
GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT OCT GAG GCG CCG GCG
Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala

747 762 777
AGC CCG GCG AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile

792 807 822 837
 OCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC
 5 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile

852 867 882 897
 CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG GTG
 10 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val

912 927 942
 GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT
 15 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val

957 972 987 1002
 ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp

1017 1032 1047
 20 ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro

1062 1077 1092 1107
 25 GCG GTC CTT CGC TGG AOC CGG GAG AAG CAG OCA AAC TAT GGG CTA GOC ATT GAG
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu

1122 1137 1152 1167
 30 GTG ACT CAC CTC CAT CAG ACT CGG AOC CAC CAG GGC CAG CAT GTC AGG ATT AGC
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

1182 1197 1212
 CGA TCG TTA CCT CAA GGG AGT GGG AAT TGG GOC CAG CTC CGG CCC CTC CTG GTC
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val

1227 1242 1257 1272
 35 ACC TTT GGC CAT GAT GGC CGG GGC CAT GOC TTG AOC CGA CGC CGG AGG GOC AAG
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Ala Lys

1287 1302 1317
 40 CGT AGC CCT AAG CAT CAC TCA CAG CGG GOC AGG AAG AAG AAT AAG AAC TGC CGG
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg

1332 1347 1362 1377
 45 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val

1392 1407 1422 1437
 50 GOC CCA CCA GGC TAC CAG GOC TTC TAC TGC CAT GGC GAC TGC CCC TTT CCA CTG
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu

1452 1467 1482
 55 GCT GAC CAC CTC AAC TCA ACC AAC CAT GOC ATT GTG CAG ACC CTG GTC AAT TCT
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

1497 1512 1527 1542
 GTC AAT TOC AGT ATC CCC AAA GOC TGT TGT GTG CCC ACT GAA CTG AGT GOC ATC
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
 5
 1557 1572 1587
 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
 10
 1602 1617 (408) 1636 1646 1656
 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGTCAGG CAGTCCTTGA GGATAGACAG
 MET Val Val Glu Gly Cys Gly Cys Arg
 15
 1666 1676 1686 1696 1706
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA
 20
 1716 1726 1736 1746 1756
 TCCACTCACC CACACACTAC ACAGACTGCT TCCTTATAGC TGGACTTTTA
 25
 1766 1776 1786 1796 1806
 TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC ATTCACCTTG
 30
 1816 1826 1836 1846 1856
 ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA
 35
 1866 1876 1886 1896 1906
 TTTTGACAAA ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG
 40
 1916 1926 1936 1946
 AGTCATTATT TTAAAAAAA AAAAAAACT CTAGAGTCGA CGGAATTC
 45

12. Gen, das menschliches BMP-4 codiert, das die in Anspruch 11 angegebene Aminosäuresequenz aufweist.

13. Gen, das ein Protein codiert, das Eigenschaften von BMP-4 zeigt, und eine DNA-Sequenz umfaßt, die:

(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 11 unterscheidet;

(b) mit einer DNA-Sequenz nach Anspruch 11 oder nach vorstehendem Absatz (a) hybridisiert; oder

(c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 11 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht.

14. DNA-Sequenz nach Anspruch 13, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.

15. DNA-Sequenz nach Anspruch 13, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.

16. Vektor, enthaltend das Gen oder die DNA-Sequenz nach einem der Ansprüche 1 bis 15 in einer funktionellen Verbindung mit einer Expressions-Kontrollsequenz.

17. Zelle, dadurch gekennzeichnet, daß sie mit einem Vektor nach Anspruch 16 transformiert ist.

18. Zelle nach Anspruch 17, dadurch gekennzeichnet, daß sie eine Säugerzelle, eine Bakterienzelle, eine Insektenzelle oder eine Hefezelle ist.

19. Zelle nach Anspruch 18, dadurch gekennzeichnet, daß sie eine CHO-Zelle ist.

20. Protein, das Eigenschaften von BMP-2 aufweist, das durch ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 1 bis 10 codiert ist.

21. Protein, das Eigenschaften von BMP-2 aufweist, das erhältlich ist durch die Schritte

- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Vektor ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 1 bis 10 umfaßt, und
- Gewinnen des Proteins aus dem Kulturmedium.

22. Protein, das Eigenschaften von BMP-4 aufweist, das durch ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 11 bis 15 codiert ist.

23. Protein, das Eigenschaften von BMP-4 aufweist, das erhältlich ist durch die Schritte

- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Vektor ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 11 bis 15 umfaßt und
- Isolieren des Proteins aus dem Kulturmedium.

24. Verfahren zur Herstellung des Proteins nach Anspruch 21 oder 23, umfassend die Schritte

- Züchten der Zelle nach Anspruch 17 in einem geeigneten Kulturmedium und
- Gewinnen des Proteins aus dem Kulturmedium.

25. Arzneimittel, dadurch gekennzeichnet, daß es, einzeln oder in Kombination, die Proteine nach einem der Ansprüche 20 bis 23 und einen pharmakologisch verträglichen Träger umfaßt.

26. Arzneimittel nach Anspruch 25, dadurch gekennzeichnet, daß es ferner eine Matrix umfaßt, die fähig ist, das Arzneimittel an die Stelle des Knochen- oder Knorpelschadens zu liefern und eine Struktur zur Induktion der Knochen- oder Knorpelbildung bereitzustellen.

27. Arzneimittel nach Anspruch 26, dadurch gekennzeichnet, daß die Matrix Hydroxyapatit, Kollagen, Polyessigsäure oder Tricalciumphosphat umfaßt.

28. Verwendung des Proteins nach einem der Ansprüche 20 bis 23, einzeln oder in Kombination, zur Herstellung eines Arzneimittels zur Induktion der Knochen- oder Knorpelbildung.

Patentansprüche für folgenden Vertragsstaat : AT

1. Verfahren zur Herstellung eines menschlichen BMP-2 codierenden Gens, das die nachfolgende DNA-Sequenz umfaßt:

10 20 30 40 50
 GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG
 5 60 70 80 90 100
 CAGGGAGAAT AACTTGCGCA CCCCACTTTG CGCCGGTGCC TTTGCCCCAG
 110 120 130 140 150
 CGGAGCCTGC TTCGCCATCT CCGAGCCCCA CCGCCCCTCC ACTCCTCGGC
 10 160 170 180 190 200
 CTTGCCCCGAC ACTGAGACGC TGTTCCCAGC GTGAAAAGAG AGACTGCGCG
 210 220 230 240 250
 15 GCCGGCACCC GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTCCGT
 260 270 280 290 300
 CCTTGCGCCA GGTCCCTTGA CCAGAGTTT TCCATGTGGA CGCTCTTTCA
 20 310 320 330 340 350
 ATGGACGTGT CCCC CGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT
 (1) 370 385 400
 25 OGACC ATG GTG GGC GGG ACC GGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val
 415 430 445
 30 CTC CTG GGC GGC GCG GGC CTC GTT CCG GAG CTG GGC GGC AGG AAG TTC GCG
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala
 460 475 490 505
 GCG GCG TCG TCG GGC GGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu
 35 520 535 550 565
 TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC AOC CCC AGC
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser
 40 580 595 610
 AGG GAC GGC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CCG AGG CAC TCG GGT
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly
 45
 50
 55

625 640 655 670
 CAG CCG GGC TCA CCG GGC CCA GAC CAC CCG TTG GAG AGG GCA GGC AGC OGA GGC
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala
 5
 685 700 715
 AAC ACT GTG CCG AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr
 10
 730 745 760 775
 AGT GGG AAA ACA AOC CCG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu
 15
 790 805 820 835
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala
 20
 850 865 880
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys
 25
 895 910 925 940
 OCT GCA ACA GGC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu
 30
 955 970 985
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET
 1000 1015 1030 1045
 CCG TGG ACT GCA CAG GGA CAC GGC AAC CAT GGA TTC GTG GTG GAA GTG GGC CAC
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His
 35
 1060 1075 1090 1105
 TTG GAG GAG AAA CAA CGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu
 40
 1120 1135 1150
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly
 45
 1165 1180 1195 1210
 CAT GAT GGA AAA GGG CAT OCT CTC CAC AAA AGA GAA AAA CGT CAA GGC AAA CAC
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His
 50
 1225 1240 1255
 AAA CAG CCG AAA CCG CTT AAG TCC AGC TGT AAG AGA CAC OCT TTG TAC GTG GAC
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp
 1270 1285 1300 1315
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GGC
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala
 55
 1330 1345 1360 1375
 TTT TAC TGC CAC GGA GAA TGC OCT TTT OCT CTG GCT GAT CAT CTG AAC TCC ACT
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
 1390 1405 1420
 AAT CAT GGC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT OCT AAG
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

1435 1450 1465 1480
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu
 5
 1495 1510 1525
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly
 10
 1540(396) 1553 1563 1573 1583 1593 1603
 TGT CGC TAGTACAGCA AAATTAAATA CATAATATA TATATATATA TATATTTTAG AAAAAAGAAA
 Cys Arg

AAAA,

wobei das Verfahren die nachfolgenden Schritte umfaßt:

- (a) Absuchen einer Genbank durch Hybridisieren mit einem markierten bBMP-2-Fragment, wobei die Genbank aus einer von U-2 OS abgeleiteten DNA oder cDNA konstruiert war,
- (b) Isolieren positiver Clone und
- (c) Isolieren der DNA-Insertionen aus diesen Clonen.
2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Gen menschliches BMP-2 codiert, das die in Anspruch 1 angegebene Aminosäuresequenz aufweist.
3. Verfahren zur Herstellung eines Gens, das ein Protein codiert, das Eigenschaften von menschlichem BMP-2 zeigt, und eine DNA-Sequenz umfaßt, die:
 - (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 1 unterscheidet;
 - (b) mit einer DNA-Sequenz nach Anspruch 1 oder nach vorstehendem Absatz (a) hybridisiert; oder
 - (c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 1 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht,
- wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.
4. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.
5. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.
6. Verfahren zur Herstellung eines Rinder-BMP-2 codierenden Gens, umfassend die nachfolgende DNA-Sequenz:

(1)

5 GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
G H D G K G H P L H R R E K R

10 CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG
Q A K H K Q R K R L K S S C K

15 TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG
W I V A P P G Y H A F Y C H G

20 GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
E C P F P L A D H L N S T N H

25 GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC
A I V Q T L V N S V N S K I P

30 AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG
K A C C V P T E L S A I S M L

35 TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC
Y L D E N E K V V L K N Y O D

ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAATA
M V V E G C G C R

40 TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC

45 ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA

AAGAAAAACA CAGCTATTTT GAAAACATA TTTATATCTA CCGAAAAGAA

50 GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT

wobei das Verfahren die nachfolgenden Schritte umfaßt:

- 55 (a) Absuchen einer Genbank mit einer markierten auf der Grundlage der Aminosäuresequenz eines Fragmen-
tes von bBMP-2 entworfenen Sonde, wobei die Genbank aus Rinderleber-DNA oder cDNA konstruiert wurde,
- (b) Isolieren positiver Clone und

(c) Isolieren der DNA-Insertionen aus diesen Clonen.

7. Verfahren nach Anspruch 6, dadurch gekennzeichnet, daß das Gen Rinder-BMP-2 codiert, das die Aminosäuresequenz von Anspruch 6 aufweist.

8. Verfahren zur Herstellung eines Genes, das ein Protein codiert, das Eigenschaften von Rinder-BMP-2 zeigt, und DNA-Sequenzen umfaßt, die:

(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 7 unterscheiden;

(b) mit einer DNA-Sequenz nach Anspruch 7 oder nach vorstehendem Absatz (a) hybridisieren; oder

(c) Fragmente, allelische oder andere Variationen einer DNA-Sequenz nach Anspruch 7 darstellen, unabhängig davon, ob die Variationen zu Änderungen in der Peptidsequenz führen oder nicht,

wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.

9. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.

10. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.

11. Verfahren zur Herstellung eines menschlichen BMP-4 codierenden Genes, das die nachfolgende DNA-Sequenz umfaßt:

10	20	30	40	50
CTCTAGAGGG	CAGAGGAGGA	GGGAGGGAGG	GAAGGAGCGC	GGAGCCCCGGC
60	70	80	90	100
CCGGAAGCTA	GGTGAGTGTG	GCATCCGAGC	TGAGGGACGC	GAGCCTGAGA
110	120	130	140	150
CGCCGCTGCT	GCTCCGGCTG	AGTATCTAGC	TTGTCTCCCC	GATGGGATTTC
160	170	180	190	200
CCGTCCAAGC	TATCTCGAGC	CTGCAGCGCC	ACAGTCCCCG	GCCCTCGCCC
210	220	230	240	250
AGGTTCAC TG	CAACCGTTCA	GAGGTCCCCA	GGAGCTGCTG	CTGGCGAGCC
260	270	280	290	300
CGCTACTGCA	GGGACCTATG	GAGCCATTCC	GTAGTGCCAT	CCCAGCAAC
310	320	330	340	350
GCACTGCTGC	AGCTTCCCTG	AGCCTTTCCA	GCAAGTTTGT	TCAAGATTGG
360	370	380	390	400
CTGTCAAGAA	TCATGGACTG	TTATTATATG	CCTTGTTTTTC	TGTCAAGACA

(1)
CC ATG ATT CCT
MET Ile Pro

5

10

15

20

25

30

35

40

45

50

55

417 432 447 462
GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG
Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala

477 492 507
AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GGC GAG ATT CAG
Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln

522 537 552 557
GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CCG GAC TTC
Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe

582 597 612 627
GAG GCG ACA CTT CTG CAG ATG TTT GCG CTG CGC CGC CGC CCG CAG OCT AGC AAG
Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys

642 657 672
AGT GGC GTC ATT CCG GAC TAC ATG CCG GAT CTT TAC CCG CTT CAG TCT GCG GAG
Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu

687 702 717 732
GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT OCT GAG CGC CCG GGC
Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala

747 762 777
AGC CCG GGC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His-Leu-Glu Asn Ile

792 807 822 837
CCA GCG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC
Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile

852 867 882 897
OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CCG CTC TTC CCG GAG CAG GTG
Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val

912 927 942
GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT
Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val

957 972 987 1002
ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp

1017 1032 1047
AAG AGA CTG GTC CAC CAC AAT GTG ACA CCG TGG GAA ACT TTT GAT GTG AGC CCT
Thr Arg Leu Val His His Asn Val Thr Arg, Trp Glu Thr Phe Asp Val Ser Pro

1062 1077 1092 1107
GGG GTC CTT CGC TGG ACC CCG GAG AAG CAG CCA AAC TAT GGG CTA GGC ATT GAG
Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu

1122 1137 1152 1167
 GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser
 5
 1182 1197 1212
 CGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GGC CAG CTC CGG CCC CTC CTG GTC
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
 10
 1227 1242 1257 1272
 ACC TTT GGC CAT GAT GGC CGG GGC CAT GGC TTG ACC CGA CGC CGG AGG GCC AAG
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys
 15
 1287 1302 1317
 CGT AGC OCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
 20
 1332 1347 1362 1377
 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
 25
 1392 1407 1422 1437
 GGC CCA CCA GGC TAC CAG GGC TTC TAC TGC CAT GGC GAC TGC CCC TTT CCA CTG
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asn Cys Pro Phe Pro Leu
 30
 1452 1467 1482
 GGT GAC CAC CTC AAC TCA ACC AAC CAT GGC ATT GTG CAG ACC CTG GTC AAT TCT
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser
 35
 1497 1512 1527 1542
 GTC AAT TCC AGT ATC CCC AAA GGC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
 40
 1557 1572 1587
 TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
 Ser Met Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
 45
 1602 1617 (408) 1636 1646 1656
 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCCTGA GGATAGACAG
 MET Val Val Glu Gly Cys Gly Cys Arg
 50
 1666 1676 1686 1696 1706
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA
 55
 1716 1726 1736 1746 1756
 TCCACTCACC CACACACTAC ACAGACTGCT TCCTTATAGC TGGACTTTTA
 1766 1776 1786 1796 1806
 TTTAAAAAAA AAAAAAATAA AATGGAAAAA ATCCCTAAAC ATTCACCTTG

1816 1826 1836 1846 1856
ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA
5 1866 1876 1886 1896 1906
TTTTGACAAA ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG
1916 1926 1936 1946
10 AGTCATTATT TTAAAAA AAAA AAAACT CTAGAGTCGA CGGAATTC

wobei das Verfahren die nachfolgenden Schritte umfaßt:

(a) Absuchen einer Genbank durch Hybridisieren mit einem markierten bBMP-2-Fragment, wobei die Genbank aus einer von U-2 OS abgeleiteten DNA oder cDNA konstruiert war,

(b) Isolieren positiver Clone und

(c) Isolieren der DNA-Insertionen aus diesen Clonen.

12. Verfahren nach Anspruch 11, dadurch gekennzeichnet, daß das Gen menschliches BMP-4 codiert, das die in Anspruch 11 angegebene Aminosäuresequenz aufweist.

13. Verfahren zur Herstellung eines Genes, das ein Protein codiert, das Eigenschaften von BMP-4 zeigt, und eine DNA-Sequenz umfaßt, die:

(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 11 unterscheidet;

(b) mit einer DNA-Sequenz nach Anspruch 11 oder vorstehendem Absatz (a) hybridisiert; oder

(c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 11 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht,

wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.

14. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.

15. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.

16. Vektor, enthaltend das Gen oder die DNA-Sequenz nach einem der Ansprüche 1 bis 15 in einer funktionellen Verbindung mit einer Expressions-Kontrollsequenz.

17. Zelle, dadurch gekennzeichnet, daß sie mit einem Vektor nach Anspruch 16 transformiert ist.

18. Zelle nach Anspruch 17, dadurch gekennzeichnet, daß sie eine Säugerzelle, eine Bakterienzelle, eine Insektenzelle oder eine Hefezelle ist.

19. Zelle nach Anspruch 18, dadurch gekennzeichnet, daß sie eine CHO-Zelle ist.

20. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-2 zeigt, umfassend die Schritte

- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Expressionsvektor ein Gen oder eine DNA-Sequenz umfaßt, die nach einem der Ansprüche 1 bis 10 hergestellt wurden, und

- Gewinnen des Proteins aus dem Kulturmedium.

21. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-4 zeigt, umfassend die Schritte

- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Expressionsvektor ein Gen oder eine DNA-Sequenz umfaßt, die nach einem der Ansprüche 11 bis 15 hergestellt wurden, und

5 - Gewinnen des Proteins aus dem Kulturmedium.

22. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-2 oder BMP-4 zeigt, umfassend die Schritte

- Züchten der Zelle nach Anspruch 17 in einem geeigneten Kulturmedium und

- Isolieren des Proteins aus dem Kulturmedium.

23. Verfahren zur Herstellung eines Arzneimittels, dadurch gekennzeichnet, daß es ein Kombinieren der nach einem der Ansprüche 20 bis 22 hergestellten Proteine, einzeln oder in Kombination, mit einem pharmakologisch verträglichen Träger umfaßt.

24. Verfahren nach Anspruch 23, dadurch gekennzeichnet, daß das Arzneimittel ferner eine Matrix umfaßt, die fähig ist, das Arzneimittel an die Stelle des Knochen- oder Knorpelschadens zu liefern und eine Struktur zur Induktion der Knochen- oder Knorpelbildung bereitzustellen.

25. Verfahren nach Anspruch 24, dadurch gekennzeichnet, daß die Matrix Hydroxyapatit, Kollagen, Polyessigsäure oder Tricalciumphosphat umfaßt.

26. Verwendung eines Proteins nach einem der Ansprüche 20 bis 22, einzeln oder in Kombination, zur Herstellung eines Arzneimittels zur Induktion der Knochen- oder Knorpelbildung.

Revendications

Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Gène codant pour la BMP-2 humaine comprenant la séquence d'ADN suivante :

10 20 30 40 50 60 70
 GTGACTCTA GAGTGTGTG CAGCACTTG CTGGGGACTT CTTGAACITG CAGGGAGAAT AACTTGCCA
 5
 80 90 100 110 120 130 140
 CCCCACITTG CGCGGGTGGC TTGCGCCAG CCGAGCGTGC TTGCGCATCT CCGAGCCCCA CCGCGCCCTCC
 10
 150 160 170 180 190 200 210
 ACTGCTGGC CTTGCGCGAC ACTGAGCGC TGTTCGCGC GTGAAAAGAG AGACTGCGCG CGCGGCACC
 15
 220 230 240 250 260 270 280
 GCGAGAGGA GCGGGCAAAG AAAAGGAAAG GACATTGGT CCGTGGCGCA GGTGCTTTGA CCGAGTTTT
 20
 290 300 310 320 330 340 350
 TCGATGTGA CGCTTTTCA ATGGAAGTGT CCGCGGTGC TTCTAGAGC GACTGGGTC TCGTAAGGT
 25
 (1) 370 385 400
 CGACC ATG GTG GGC ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val
 30
 415 430 445
 CTC CTG GGC GGC GGC GGT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GGC
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala
 35
 450 475 490 505
 GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu
 40
 520 535 550 565
 TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser
 45
 580 595 610
 AGG GAC GGC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CCG AGG CAC TCG GGT
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly
 50
 625 640 655 670
 CAG CCG GGC TCA CCC GGC CCA GAC CAC CCG TTG GAG AGG GCA GGC AGC CGA GGC
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala
 55
 685 700 715
 AAC ACT GTG CCG AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA AGC
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr

730 745 760 775
 AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu
 5
 790 805 820 835
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala
 10
 850 865 880
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys
 15
 895 910 925 940
 OCT GCA ACA GGC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu
 20
 955 970 985
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET
 25
 1000 1015 1030 1045
 CGG TGG ACT GCA CAG GGA CAC GGC AAC CAT GGA TTC GTG GTG GAA GTG GGC CAC
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His
 30
 1060 1075 1090 1105
 TTG CAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu
 35
 1120 1135 1150
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly
 40
 1155 1180 1195 1210
 CAT GAT GGA AAA GGG CAT OCT CTC CAC AAA AGA GAA AAA CGT CAA GGC AAA CAC
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His
 45
 1225 1240 1255
 AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp
 50
 1270 1285 1300 1315
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GGC
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala
 55
 1330 1345 1360 1375
 TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
 1390 1405 1420
 AAT CAT GGC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT CCT AAG
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

1435 1450 1465 1480
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu
 5
 1495 1510 1525
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly
 10
 1540(396) 1553 1563 1573 1583 1593 1603
 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA
 Cys Arg

15 AAAA

2. Gène codant pour la BMP-2 humaine comportant la séquence d'acides aminés donnée à la revendication 1.
3. Gène codant pour une protéine montrant des propriétés de la BMP-2 humaine et comprenant une séquence d'ADN :
 - (a) qui diffère d'une séquence d'ADN de la revendication 1 dans la séquence de codons du fait de la dégénérescence du code génétique ;
 - (b) qui s'hybride avec une séquence d'ADN de la revendication 1 ou du paragraphe (a) ci-dessus ; ou
 - (c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 1, que cette variation résulte de changements dans la séquence peptidique ou non.
4. Séquence d'ADN suivant la revendication 3, qui est une séquence d'ADN génomique.
5. Séquence d'ADN suivant la revendication 3, qui est une séquence d'ADNc.
6. Gène codant pour la BMP-2 bovine comprenant la séquence d'ADN suivante :

(1) 15 30 45
 5 GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
 G H D G K G H P L H R R E K R

60 75 90
 10 CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG
 Q A K H K Q R K R L K S S C K

105 120 135
 15 AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC
 R H P L Y V D F S D V G W N D

150 165 180
 TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG
 W I V A P P G Y H A F Y C H G

195 210 225
 20 GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
 E C P F P L A D H L N S T N H

240 255 270
 25 GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC
 A I V Q T L V N S V N S K I P

385 300 315
 30 AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG
 K A C C V P T E L S A I S M L

330 345 360
 TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC
 Y L D E N E K V V L K N Y Q D

375 (129) 397 407
 35 ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAATA
 M V V E G C G C R

417 427 437 447 457
 40 TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC

467 477 487 497 507
 ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA

517 527 537 547 557
 45 AAGAAAAACA CAGCTATTTT GAAACTATA TTTATATCTA CCGAAAAGAA

567 577 587
 50 GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT

7. Gène codant pour la BMP-2 bovine contenant la séquence d'acides aminés de la revendication 6.

8. Gène codant pour une protéine montrant des propriétés de la BMP-2 bovine et comprenant des séquences d'ADN :

(a) qui diffèrent d'une séquence d'ADN de la revendication 7 dans la séquence des codons du fait de la dégénérescence du code génétique ;

(b) qui s'hybrident avec une séquence d'ADN de la revendication 7 ou du paragraphe (a) ci-dessus ; ou
(c) représentent des fragments, des variations alléliques ou autres d'une séquence d'ADN de la revendication 7, que ces variations résultent de changements dans la séquence peptidique ou non.

9. Séquence d'ADN suivant la revendication 8, qui est une séquence d'ADN génomique.

10. Séquence d'ADN suivant la revendication 8, qui est une séquence d'ADNc.

11. Gène codant pour la BMP-4 humaine comprenant la séquence d'ADN suivante :

```

10      10      20      30      40      50      60      70
      CTCTAGAGGG CAGAGGAGGA GGGAGGGGAGG GAGGAGAGCG GGAGGCGGCGC CCGGAGGCTA GGTGAGTGTG

15      80      90     100     110     120     130     140
      GCATGCGAGC TGAGGGAGGC GAGGCTGAGA CGGGCTGCTT GCTCGGGCTG AGTATCTAGC TTGTCTGCGC

20     150     160     170     180     190     200     210
      GATGGGATTC CCGTCCAGAC TATCTGAGC CTGCAGGCGC ACAGTCCCGG GCGCTGCGCC AGGTTCACIG

25     220     230     240     250     260     270     280
      CAACGTTTCA GAGGTGCGCA GGAGCTGCTG CTGGGAGAGC CGCTACTGCA GGGACCTATG GAGCCATTCC

30     290     300     310     320     330     340     350
      GATGTCGAT CCGGAGCAAC GCATCTGCTC AGCTTCCGCT AGCTTTTCCA GCAAGTTTGT TCAGATTGG

35     360     370     380     390     400     (1)
      CTGTCAAGAA TCATGGACTG TTATTTATATG CCGTGTTTTC TGTCAGACA CC ATG ATT CCT
                                .MET.Ile Pro..

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417 432 447 462
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala
 5
 477 492 507
 AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln
 10
 522 537 552 567
 GGC CAC GCG GGA GGA GCG CTC TCA GGG CAG AGC CAT GAG CTC CTG GCG GAC TTC
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe
 15
 582 597 612 627
 GAG GCG ACA CTT CTG CAG ATG TTT GCG CTG GCG GCG GCG CCG CAG OCT AGC AAG
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys
 20
 642 657 672
 AGT GCC GTC ATT CCG GAC TAC ATG GCG GAT CTT TAC GCG CTT CAG TCT GCG GAG
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu
 25
 687 702 717 732
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT OCT GAG GCG CCG GCC
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala
 30
 747 762 777
 AGC GCG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile
 35
 792 807 822 837
 CCA GCG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile
 40
 852 867 882 897
 OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT GCG CTC TTC CCG GAG CAG GTG
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val
 45
 912 927 942
 GAC CAG GCG OCT GAT TGG GAA AGG GCG TTC CAC CGT ATA AAC ATT TAT GAG GTT
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val
 50
 957 972 987 1002
 ATG AAG CCC CCA GCA GAA GTG GTG OCT GCG CAC CTC ATC ACA CCA CTA CTG GAC
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp
 55
 1017 1032 1047
 ACG AGA CTG GTC CAC CAC AAT GTG ACA CCG TGG GAA ACT TTT GAT GTG AGC CCT
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro
 1052 1077 1092 1107
 GCG GTC CTT CCG TGG ACC CCG GAG AAG CAG CCA AAC TAT GCG CTA GCC ATT GAG
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu
 1122 1137 1152 1167
 GTG ACT CAC CTC CAT CAG ACT GCG ACC CAC CAG GCG CAG CAT GTC AGG ATT AGC
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

1182 1197 1212
 CGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
 5
 1227 1242 1257 1272
 ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG AOC CGA CGC CGG AGG GCC AAG
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys
 10
 1287 1302 1317
 CGT AGC OCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
 1332 1347 1362 1377
 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
 15
 1392 1407 1422 1437
 GCC CCA CCA GGC TAC CAG GGC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asn Cys Pro Phe Pro Leu
 20
 1452 1467 1482
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser
 25
 1497 1512 1527 1542
 GTC AAT TOC AGT ATC CCC AAA GGC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
 1557 1572 1587
 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
 30
 1602 1617 (408) 1636 1646 1656
 ATG GTA GTA GAG GGA TGT GGG TGC GGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
 MET Val Val Glu Gly Cys Gly Cys Arg
 35
 1666 1676 1686 1696 1706 1716 1726
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CAGCTTCCCA TOCACTCAAC CACACACTAC
 40
 1736 1746 1756 1766 1776 1786 1796
 ACAGACTGCT TOCTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAATAA AATGGAAAAA ATCCCTAAAC
 45
 1806 1816 1826 1836 1846 1856 1866
 ATTCACTTIG AACTATTTA TGACTTTACG TGCATTTGT TTAACCTAT TGAATATATA TTTCACAAA
 1876 1886 1896 1906 1916 1926 1936
 ATATATTTAT AACCTAGTAT TAAAGAAAAA AATTAATATG AGTCATTTAT TTAAAAAATA AAAAAAAT
 50
 1946
 CTAGAGTGA CGGAATTC
 55

12. Gène codant pour la BMP-4 humaine comportant la séquence d'acides aminés donnée à la revendication 11.

13. Gène codant pour une protéine montrant des propriétés de la BMP-4 et comprenant une séquence d'ADN :

(a) qui diffère d'une séquence d'ADN de la revendication 11 dans la séquence des codons du fait de la dégénérescence du code génétique ;

(b) qui s'hybride avec une séquence d'ADN de la revendication 11 ou du paragraphe (a) ci-dessus ; ou

(c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 11, que cette variation résulte de changements dans la séquence peptidique ou non.

14. Séquence d'ADN suivant la revendication 13, qui est une séquence d'ADN génomique.

15. Séquence d'ADN suivant la revendication 13, qui est une séquence d'ADNc.

16. Vecteur contenant le gène ou la séquence d'ADN suivant l'une quelconque des revendications 1 à 15, en association active avec une séquence de contrôle d'expression.

17. Cellule transformée avec un vecteur de la revendication 16.

18. Cellule suivant la revendication 17, qui est une cellule mammifère, une cellule bactérienne, une cellule d'insecte ou une cellule de levure.

19. Cellule suivant la revendication 18, qui est une cellule CHO.

20. Protéine montrant des propriétés de la BMP-2, qui est codée par un gène ou une séquence d'ADN de l'une quelconque des revendications 1 à 10.

21. Protéine montrant des propriétés de la BMP-2, qui est obtenable par les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN de l'une quelconque des revendications 1 à 10, et de récupération de ladite protéine du milieu de culture précité.

22. Protéine montrant des propriétés de la BMP-4, qui est codée par un gène ou une séquence d'ADN de l'une quelconque des revendications 11 à 15.

23. Protéine montrant des propriétés de la BMP-4, qui est obtenable par les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN de l'une quelconque des revendications 11 à 15, et de récupération de ladite protéine du milieu de culture précité.

24. Procédé de production de la protéine suivant l'une ou l'autre des revendications 21 et 23, comprenant les étapes de culture dans un milieu de culture approprié de la cellule de la revendication 17 et d'isolement de ladite protéine du milieu de culture précité.

25. Composition pharmaceutique comprenant les protéines de l'une quelconque des revendications 20 à 23, individuellement ou en combinaison, et un véhicule pharmaceutiquement acceptable.

26. Composition pharmaceutique suivant la revendication 25, comprenant de plus une matrice pouvant distribuer la composition au site de l'anomalie osseuse ou cartilagineuse et formant une structure pour induire une formation osseuse ou cartilagineuse.

27. Composition pharmaceutique suivant la revendication 26, dans laquelle ladite matrice comprend de l'hydroxyapatite, du collagène, de l'acide polylactique ou du phosphate tricalcique.

28. Utilisation d'une protéine suivant l'une quelconque des revendications 20 à 23, individuellement ou en combinaison, pour la préparation d'une composition pharmaceutique pour induire une formation osseuse ou cartilagineuse.

Revendications pour l'Etat contractant suivant : AT

1. Procédé de préparation d'un gène codant pour la BMP-2 humaine comprenant la séquence d'ADN suivante :

EP 0 313 578 B1

10 20 30 40 50 60 70
GTGACTCTA GAGTGTGTGT CAGCCTTGG CTGGGGACTT CTGGAACITG CAGGGAGAAT AACITGGCA

5 80 90 100 110 120 130 140
CCCCACITTG CGCGGTGOC TTGGOOCAG CGGAGCCTGC TTGOCATCT CCGAGOOCA CGGGOOCCTC

10 150 160 170 180 190 200 210
ACTOCTGGC CTGGOOCAG ACTGGAOCG TGTTCCAGC GTGAAAAGAG AGACTGGOG GCGGCAACC

15 220 230 240 250 260 270 280
GGGAGAAGGA GGAGGCAAG AAAAGGAOC GACATTOGGT CCTTGGCCA GGTCTTTGA CCAGATTTT

20 290 300 310 320 330 340 350
TCCATGTGA CGCTCTTCA ATGGAOGTGT CCGCGCTGC TTCTTAGAC GACTGGGTC TCCFAAGGT

(1) 370 385 400
CGAC ATG GTG GOC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCG CAG GTC
MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val

25 415 430 445
CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GCG
Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala

30 460 475 490 505
GCG GCG TCG TCG GGC CGC CCG TCA TCC CAG CCG TCT GAC GAG GTC CTG AGC GAG
Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu

35 520 535 550 565
TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCG ACC CCG AGC
Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser

40 580 595 610
AGG GAC GOC GTG GTG CCG CCG TAC ATG CTA GAC CTG TAT CGC AGG CAC TCG GGT
Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly

45 625 640 655 670
CAG CCG GGC TCA CCG GCG CCA GAC CAC CCG TTG GAG AGG CCA GCG AGC CGA GCG
Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala

50 685 700 715
AAC ACT GTG CGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG
Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr

55

730 745 760 775
 AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu
 5
 790 805 820 835
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala
 10
 850 865 880
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys
 895 910 925 940
 15 OCT GCA ACA GGC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu
 955 970 985
 20 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET
 1000 1015 1030 1045
 CCG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His
 25 1060 1075 1090 1105
 TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu
 1120 1135 1150
 30 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GCC
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly
 1165 1180 1195 1210
 35 CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His
 1225 1240 1255
 AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp
 40 1270 1285 1300 1315
 TTC AGT CAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala
 1330 1345 1360 1375
 45 TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
 1390 1405 1420
 50 AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT CCT AAG
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys
 55

1435 1450 1465 1480
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GGT ATC TCG ATG CTG TAC CTT GAC GAG
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu
 5
 1495 1510 1525
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG CAC ATG GTT GTG GAG GGT TGT GGG
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly
 10
 1540(396) 1553 1563 1573 1583 1593 1603
 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA
 Cys Arg

15 AAAA ,

dans lequel ledit procédé comprend les étapes suivantes

- 20 a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant de U-2 OS avec un fragment de BMP-2 marqué par hybridation,
 b) l'isolement des clones positifs, et
 c) l'isolement des inserts d'ADN de ces clones.
- 25 2. Procédé suivant la revendication 1, dans lequel le gène code pour la BMP-2 humaine ayant la séquence d'acides aminés donnée à la revendication 1.
- 30 3. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-2 humaine et comprenant une séquence d'ADN :
- 35 a) qui diffère d'une séquence d'ADN de la revendication 1 dans la séquence des codons du fait de la dégénérescence du code génétique ;
 b) qui s'hybride avec une séquence d'ADN de la revendication 1 ou du paragraphe (a) ci-dessus ; ou
 c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 1, que cette variation résulte de changements dans la séquence peptidique ou non,
- dans lequel le procédé susdit comprend des techniques standards de biologie moléculaire.
4. Procédé suivant la revendication 3, dans lequel la séquence d'ADN est une séquence d'ADN génomique.
- 40 5. Procédé suivant la revendication 3, dans lequel la séquence d'ADN est une séquence d'ADNc.
- 45 6. Procédé de préparation d'un gène codant pour la BMP-2 bovine comprenant la séquence d'ADN suivante :
- 50
- 55

(1) 15 30 45
 GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
 G H D G K G H P L H R R E K R
 5
 60 75 90
 CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG
 Q A K H K Q R K R L K S S C K
 10
 105 120 135
 AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC
 R H P L Y V D F S D V G W N D
 15
 150 165 180
 TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG
 W I V A P P G Y H A F Y C H G
 195 210 225
 GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
 E C P F P L A D H L N S T N H
 20
 240 255 270
 GGC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC
 A I V Q T L V N S V N S K I P
 25
 385 300 315
 AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG
 K A C C V P T E L S A I S M L
 30
 330 345 360
 TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC
 Y L D E N E K V V L K N Y Q D
 35
 375 (129) 397 407
 ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA
 M V V E G C G C R

40 417 427 437 447 457
 TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC
 467 477 487 497 507
 ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA
 45 517 527 537 547 557
 AAGAAAAACA CAGCTATTTT GAAACTATA TTTATATCTA CCGAAAAGAA
 567 577 587
 GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT,

dans lequel ledit procédé comprend les étapes suivantes :

- a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant de foie bovin avec une sonde marquée conçue sur la base de la séquence d'acides aminés d'un fragment de bBMP-2,
- b) l'isolement des clones positifs, et
- c) l'isolement des inserts d'ADN de ces clones.

7. Procédé suivant la revendication 6, dans lequel le gène code pour de la BMP-2 bovine ayant la séquence d'acides

aminés de la revendication 6.

8. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-2 bovine et comprenant des séquences d'ADN :

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a) qui diffèrent d'une séquence d'ADN de la revendication 7 dans la séquence des codons du fait de la dégénérescence du code génétique ;

b) qui s'hybrident avec une séquence d'ADN de la revendication 7 ou du paragraphe a) ci-dessus ; ou

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c) représentent des fragments, des variations alléliques ou autres d'une séquence d'ADN de la revendication 7, que ces variations résultent de changements dans la séquence peptidique ou non,

dans lequel le procédé précité comprend des techniques standards de biologie moléculaire.

9. Procédé suivant la revendication 8, dans lequel la séquence d'ADN est une séquence d'ADN génomique.

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10. Procédé suivant la revendication 8, dans lequel la séquence d'ADN est une séquence d'ADNc.

11. Procédé de préparation d'un gène codant pour la BMP-4 humaine comprenant la séquence d'ADN suivante :

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10 20 30 40 50 60 70
 CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGGCG GGAGGCGGCG CCGGAGCTA GGTGAGTGTG
 5
 80 90 100 110 120 130 140
 GCATCGGAGC TGAGGGAAGC GAGCGTGAGA CGCGCTGCT GCTCGGCTG AGTATCTAGC TTGTCTGCCC
 10
 150 160 170 180 190 200 210
 GATGGGATTC CGCTCGAGC TATCTGAGC CTGAGGCGC ACAGTCCCG GCGCTGCGC AGGTTCAGT
 15
 220 230 240 250 260 270 280
 CAACCGTTCA GAGGTCCCA GGAGCTGCTG CTGGCGAGCC CGCTACTGCA GGGACCTCTG GAGCATTC
 20
 290 300 310 320 330 340 350
 GTAGTGCAT CCGGAGCAAC GCACCTGCTC AGCTTCCCTG AGCTTTTCCA GCAAGTTTGT TCAAGATTGG
 25
 360 370 380 390 400 (1)
 CTGTCAAGAA TCATGGACTG TTATTTATG CTTTGTTC TGTCAGACA CC ATG ATT OCT
 MET Ile Pro
 30
 417 432 447 462
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala
 35
 477 492 507
 AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GGC GAG ATT CAG
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln
 40
 522 537 552 557
 GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CCG GAC TTC
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe
 45
 582 597 612 627
 GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG CCT AGC AAG
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys
 50
 642 657 672
 AGT GGC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu
 55
 687 702 717 732
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CGC GGC
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala

747 762 777
 AGC OGG GOC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile
 5
 792 807 822 837
 CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile
 10
 852 867 882 897
 OCT GAG AAC GAG GTG ATC TOC TCT GCA GAG CTT OGG CTC TTC OGG GAG CAG GTG
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val
 15
 912 927 942
 GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val
 20
 957 972 987 1002
 ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
 Met Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp
 25
 1017 1032 1047
 ACG AGA CTG GTC CAC CAC AAT GTG ACA OGG TGG GAA ACT TTT GAT GTG AGC OCT
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro
 30
 1062 1077 1092 1107
 GCG GTC CTT CGC TGG AOC CGG GAG AAG CAG CCA AAC TAT GGG CTA GGC ATT GAG
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu
 35
 1122 1137 1152 1167
 GTG ACT CAC CTC CAT CAG ACT CGG AOC CAC CAG GGC CAG CAT GTC AGG ATT AGC
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser
 40
 1182 1197 1212
 CGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GOC CAG CTC OGG CCC CTC CTG GTC
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
 45
 1227 1242 1257 1272
 ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG AOC CGA CGC OGG AGG GCC AAG
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Ala Lys
 50
 1287 1302 1317
 CGT AGC OCT AAG CAT CAC TCA CAG CGG GOC AGG AAG AAG AAT AAG AAC TGC CGG
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
 55
 1332 1347 1362 1377
 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
 1392 1407 1422 1437
 GGC CCA CCA GGC TAC CAG GGC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Glv Asn Cys Pro Phe Pro Leu
 1452 1467 1482
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GGC ATT GTG CAG ACC CTG GTC AAT TCT
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

1497 1512 1527 1542
 GTC AAT TOC AGT ATC CCC AAA GGC TGT TGT GTG CCC ACT GAA CTG AGT GGC ATC
 5 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile

 1557 1572 1587
 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
 10 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu

 1602 1617 (408) 1636 1646 1656
 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
 MET Val Val Glu Gly Cys Gly Cys Arg

 1666 1676 1686 1696 1706 1716 1726
 15 ATATACACAC CACACACACA CACCACATAC AOCACACACA CAOGTTCOCCA TOCACTCAOC CACACACTAC

 1736 1746 1756 1766 1776 1786 1796
 20 ACAGACTGCT TOCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAATAA AATGGAAAA ATCCTTAAAC

 1806 1816 1826 1836 1846 1856 1866
 25 ATTCAOCTTG AACTTATTA TGACTTTACG TGCAAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA :

 1876 1886 1896 1906 1916 1926 1936
 25 ATATATTAT AACTACGAT TAAAAGAAAA AATAAAATG AGTCATTATT TTAACAAAAA AAAAAAACT

 30 1946
 CTAGAGTOGA CGGAATTC ,

dans lequel le procédé précité comprend les étapes suivantes :

- 35 a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant d'U-2 OS avec un fragment bBMP-2 marqué par hybridation,
 b) l'isolement des clones positifs, et
 c) l'isolement des inserts d'ADN de ces clones.
- 40 12. Procédé suivant la revendication 11, dans lequel le gène code pour la BMP-4 humaine ayant la séquence d'acides aminés donnée à la revendication 11.
- 45 13. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-4 et comprenant une séquence d'ADN :
- 45 a) qui diffère d'une séquence d'ADN de la revendication 11 dans la séquence des codons du fait de la dégénérescence du code génétique ;
 b) qui s'hybride avec une séquence d'ADN de la revendication 11 ou du paragraphe a) ci-dessus ; ou
 50 c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 11, que cette variation résulte de changements dans la séquence peptidique ou non,

dans lequel le procédé précité comprend des techniques standards de biologie moléculaire.

- 55 14. Procédé suivant la revendication 13, dans lequel la séquence d'ADN est une séquence d'ADN génomique.
15. Procédé suivant la revendication 13, dans lequel la séquence d'ADN est une séquence d'ADNc.
16. Vecteur contenant le gène ou la séquence d'ADN préparé suivant l'une quelconque des revendications 1 à 15, en

association active avec une séquence de contrôle d'expression.

17. Cellule transformée avec un vecteur de la revendication 16.

5 18. Cellule suivant la revendication 17, qui est une cellule mammifère, une cellule bactérienne, une cellule d'insecte ou une cellule de levure.

19. Cellule suivant la revendication 18, qui est une cellule CHO.

10 20. Procédé de préparation d'une protéine montrant des propriétés de la BMP-2, dans lequel ledit procédé comprend les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN préparé suivant l'une quelconque des revendications 1 à 10 et de récupération de ladite protéine du milieu de culture précité.

15 21. Procédé de préparation d'une protéine montrant des propriétés de la BMP-4, dans lequel ledit procédé comprend les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN préparé suivant l'une quelconque des revendications 11 à 15 et de récupération de ladite protéine du milieu de culture précité.

20 22. Procédé de production d'une protéine montrant des propriétés de la BMP-2 ou BMP-4, comprenant les étapes de culture dans un milieu de culture approprié de la cellule de la revendication 17 et d'isolement de ladite protéine du milieu de culture précité.

25 23. Procédé de préparation d'une composition pharmaceutique comprenant la combinaison des protéines préparées suivant l'une quelconque des revendications 20 à 22, individuellement ou en combinaison avec un véhicule pharmaceutiquement acceptable.

30 24. Procédé suivant la revendication 23, dans lequel la composition pharmaceutique susdite comprend de plus une matrice pouvant distribuer la composition au site de l'anomalie osseuse ou cartilagineuse et constituer une structure pour induire une formation osseuse ou cartilagineuse.

25. Procédé suivant la revendication 24, dans lequel la matrice comprend de l'hydroxyapatite, du collagène, de l'acide polylactique ou du phosphate tricalcique.

35 26. Utilisation d'une protéine préparée suivant l'une quelconque des revendications 20 à 22, individuellement ou en combinaison, pour la préparation d'une composition pharmaceutique pour induire une formation osseuse ou cartilagineuse.

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